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Pyridoimidazolium Cationic Dyes: Theory, Synthesis, and Sub-cellular Localization

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Pyridoimidazolium Cationic Dyes: Theory, Synthesis, and Sub-cellular Localization

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Masters of Science
Chemistry

By
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Marshall University
Huntington, West Virginia
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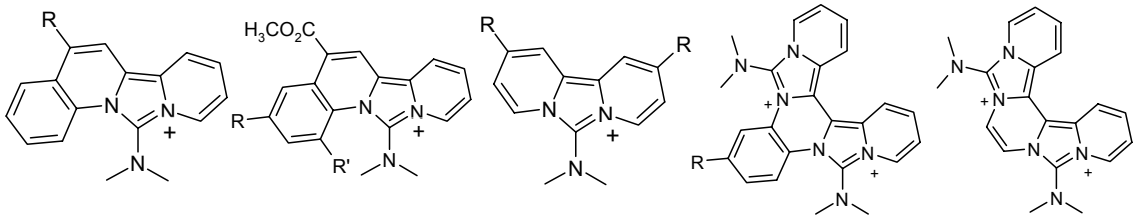
Abstract
Pyridoimidazolium Cationic Dyes: Theory, Synthesis, and Sub-cellular Localization

by: Robert William Rambacher

(PICs, Figure 1) are a new class of fluorescent dyes which are prepared by an exceedingly flexible methodology. Using computational chemical methods, the excitation and emission maxima of these dyes were simulated. This was best accomplished through geometry optimization (MM+) followed by calculating the electronic spectrum with ZINDO/S. This protocol has been found to be effective and has the potential to greatly streamline the design of new longer wavelength dyes. Several new longer wavelength dyes have now been identified as synthetic targets.

The presence of amino groups on a known fluorescent ring system is well established to markedly affect the optical properties of the dye. Methodology had been developed toward the amination of different position on the 2-(2-pyridyl)-carboxyl-quinoline ring system, leading to the testing of 3 new amino substituted fluorescent dyes, including 4-amino-2-(2-pyridyl)-quinoline. This is also the precursor to 4-isothiocyanato-2-(2-pyridyl)-quinoline, which is a potentially useful cellular tag for proteins.

The behavior of green fluorescent PICs in live smooth muscle cells was probed using confocal microscopy. Several dyes were proven to be effective in staining the mitochondria. A novel red (488/675nm) nuclear envelope stain was identified and investigated.



1a = R = CO_2H
2a = R = CO_2CH_3
3a = R = CO_2NHNH_2
4a = R = CO_2N_3
5a = R = $NHCO_2CH_2CH_3$
6a = R = NH_2
7a = R = NO_2
8a = R = NH_2
9a = R' = NO_2
10a = R' = NH_2
11b = R = CO_2CH_3
12b = R = CO_2H
13b = R = C_5H_5N
14b = R = CH_3-4-C_5H_5N
15b = R = C_6H_6
16b = R = NH_2
17c = R = CO_2H
18c = R = H
19d

Figure 1: PICs investigated in research

Acknowledgments

I would like to thank Dr. Norton for his general advice and, who during the SEM class, spawned part of my thesis. I would also like to thank Dr. Wright whose students, Ava Dykes and Chenwei Lee, supplied cells. Thanks to Dr. Frankberry and the rest of the biology department for all their help and use of their facilities. Also I would like to thank my thesis committee and the rest of the Chemistry Department. Lastly, a special thank you to Dr. Morgan for his expert tutelage in all matters.

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Chapter 1: Theory of Dyes

Introduction

Fluorescence is a phenomenon during which a molecule absorbs a photon, is raised to a high-energy state, where it remains for sometime, then returns to the ground state, emitting a photon of light. While in the high-energy state the molecule undergoes an internal conversion, which is the radiationless transition between energy states of the same spin. In a molecular system, the energy required to excite an electron into a higher energy state is always greater than the emission energy. The Jablonski diagram (Figure 1) indicates how fluorescence and phosphorescence can be produced. The difference in the excitation and the emission maxima is called the Stroke's shift (Figure 2).

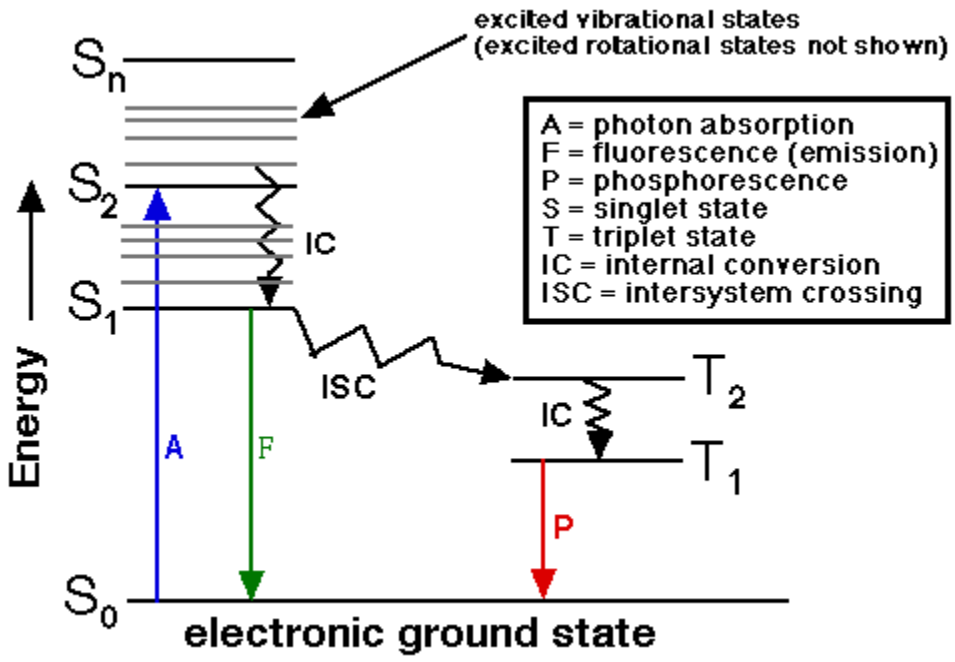


Figure 1: The Jablonski diagram

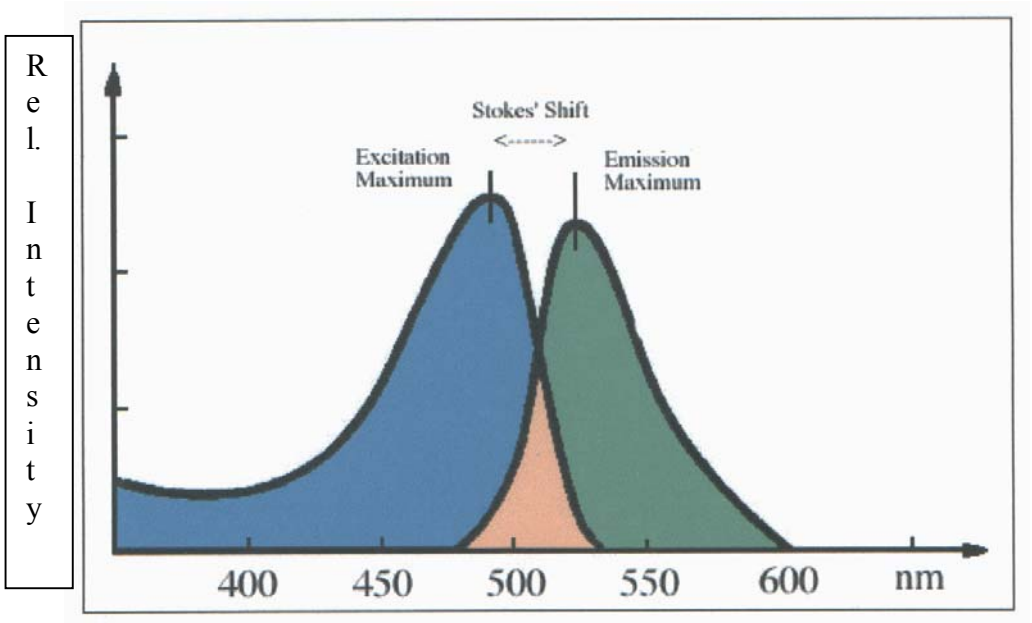
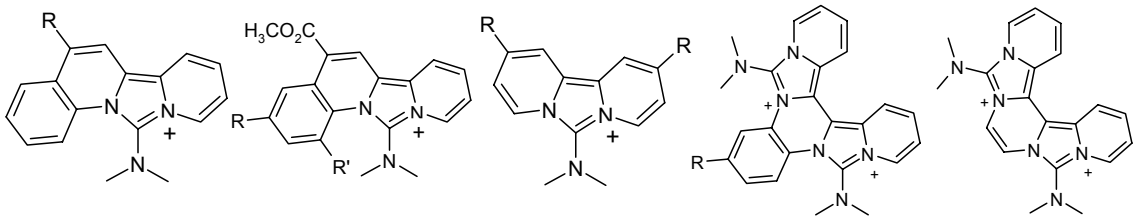


Figure 2: Stokes' shift, which is the difference between excitation and emission maxima

Considering the increasing importance and number of applications for fluorescent dyes in analytical,[1] medicinal,[2] and biochemical[3] fields, there is a demand for more specific dyes and sensors, tailored to each application. A tremendous number of pyridoimidazolium cations (PICs, Figure 3) can be synthesized using our patented approach.[1] Molecular modeling might be a convenient and effective means of identifying dyes, which would show interesting optical properties, most notably, longer wavelength fluorescence. Computational chemistry is able to predict the structures, electronic spectra and other chemical properties of molecules with ample accuracy.[4] There is a literature precedent suggesting that computer modeling offers a more systematic approach to the design of fluorescent dyes.[6]



1a = R = CO_2H **7a** = R = NO_2 **11b** = R = CO_2CH_3 **17c** = R = CO_2H **19d**
2a = R = CO_2CH_3 **8a** = R = NH_2 **12b** = R = CO_2H **18c** = R = H
3a = R = CO_2NHNH_2 **9a** = R' = NO_2 **13b** = R = C_5H_5N
4a = R = CO_2N_3 **10a** = R' = NH_2 **14b** = R = CH_3-4-C_5H_5N
5a = R = $NHCO_2CH_2CH_3$ **15b** = R = C_6H_6
6a = R = NH_2 **16b** = R =NH_2

Figure 3: PICs investigated in research

Molecular modeling can be performed using either a semi-empirical or an *ab initio* approach. The semi-empirical methods are used more frequently by practical chemists than the more expensive and complicated *ab initio* methods for the prediction of molecular properties.[4] Semi-empirical methods use parameters derived from experimental data, simplifying the approximation of the Schrodinger equation, and thus lowering the expense of the calculation. Semi-empirical calculations are reasonably accurate, can be obtained quickly, and can be applied to large or complex problems.

Many problems are simply not amenable to *ab initio* calculations because of their complexity, even when they are reduced to a minimum. For this reason semi-empirical methods were used for optimization (MM+) in this thesis. The optimization is an important element since overlooking small errors in the optimization will result in non-reliable calculations.[4]

Electronic spectra can be predicted by using molecular modeling to study excited states. There is little theoretical data on excited states available in the current literature.[5]

Modeling excited states and predicting the optical properties of molecules remains a difficult problem, due to the fact sometimes vibration states are ignored. Configuration interaction (CI) can be used to model excited states as combinations of single substitutions of the Hartree-Fock ground state.[4] This method (Configuration Interaction-singles) is described by it founders as an "adequate zeroth-order treatment of excited states of molecules."[4] CI was used to determine the absorption and fluorescence spectral properties of pyridyl-quinoline derivatives with different substitution patterns. Experimental results were complemented by theoretical calculations using semi-empirical CI molecular orbital calculations (ZINDO/S), to aid in the design of new and longer wavelength absorbing and emitting dyes.

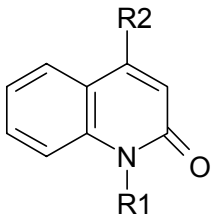
Coumarins[6](Figure 4) are a naturally occurring and easily synthesized family of fluorescent heterocycles. They have found widespread use as photosensitizers[7] and laser dyes. [8,9,10,11] Coumarins can be used to evaluate computational methodologies for the prediction of optical properties of dyes because a large amount of experimental results exist in the literature. This methodology from literature was used to study coumarins and was successful in predicting optical spectra. Fabian's[6] group determined the optical properties of these coumarins as compared to known values and was not repeated during my research. This methodology allowed for the determination of the optical properties of new coumarins before synthesis.

Table 1 summarizes the excitation data obtained by the Fabian[6] and the Zindo/s data. The electronic excitation energy is relatively insensitive to substitution. An

adequate correlation exists (Table 1) between the calculated and experimentally determined excitation energies (error of 0 to 3%) and the calculation of the emission energies (error of 0 to 7%) (Table 2). Low level *ab initio* calculations were also performed, but were less effective in modeling the electronic spectra than the combination of optimization with AM1 followed by electronic spectra simulation using ZINDO/S. The authors of the coumarin study were confident that higher levels of theory would not alter the present results, since low level *ab initio* were shown to be less effective than the semi-empirical methods. Substitution effects appear to exert a more pronounced effect on emission energies than on absorption energies.[10]

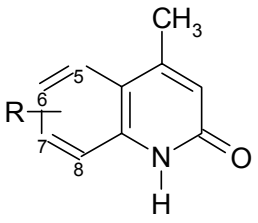
In Fabian[6] work, the excitation and emission maxima were determined with geometry optimization with AM1, followed by calculation of the transition energies using ZINDO/S. The proper treatment of the effect of hydrogen bonding is outside the scope of this approximation. Solvents were shown to have very little effect on transition energy (100 cm^{-1}).

Computational methods allow the prediction of both absorption and emission maxima with reasonable accuracy (error 0 to 7 %) in the coumarin study. This methodology should also be effective in predicting the optical properties of PICs (pyridoimidazolium cations) with ample accuracy, due to the fact of the similarity of coumarins structure as compared to PICs. Thus, these calculations provide a valuable tool in the rational design of dyes with longer wavelengths like these developed in our laboratory.



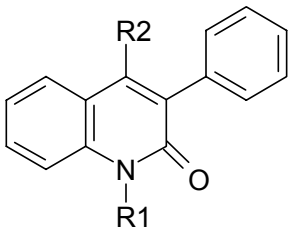
20-23

	R1	R2
20	H	H
21	CH_3	H
22	H	CH_3
23	H	C_6H_5



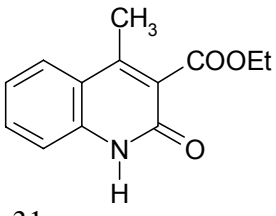
24-27

	R
24	5-CH_3O
25	6-CH_3O
26	7-CH_3O
27	8-CH_3O



28-30

	R1	R2
28	H	H
29	H	COOH
30	CH_3	Cl



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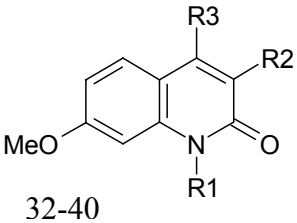
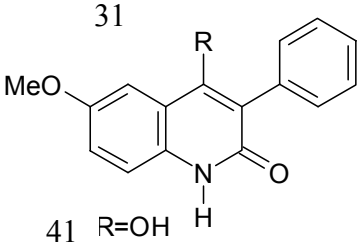


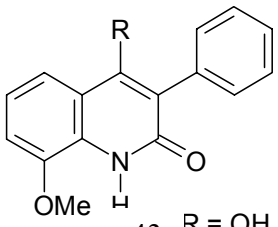
32-40

	R1	R2	R3
32	H	C_6H_5	OH
33	CH_3	C_6H_5	OH
34	C_2H_5	C_6H_5	CH_3C00
35	H	4-CH_3-O-C_6H_4	OH
36	C_2H_5	4-CH_3-O-C_6H_4	CH_3C00
37	H	C_6H_5	$C_6H_5CH_2NH$
38	H	C_6H_5	4-CH_3-C_6H_4-SO_3
39	C_2H_5	C_6H_5	Cl
40	H	4-Acetaminobenzyl	CH_3



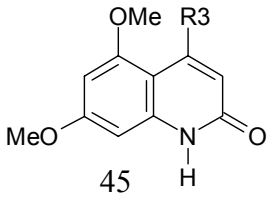
41 R=OH

42 R = $(CH_3)_2NCOO$



43 R = OH

44 R = CH_3COO



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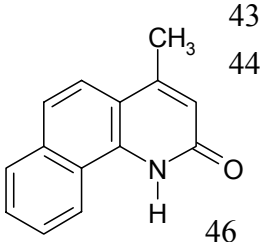

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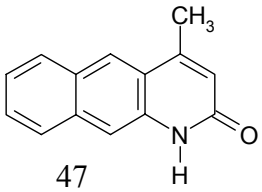

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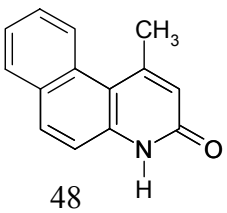

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Figure 4: Structures of coumarins

Table 1[10]: Experimental and calculated (ZINDO) absorption maxima (cm^{-1}) extinction coefficients ϵ ($1\ mol^{-1}$) and oscillator strength *f*

Table 1	Experimental and calculated (ZINDO) absorption maximum, extinction coefficient, and oscillator strengths			
Compd	υ_{exp} (cm^{-1})	ε ($1\ mol^{-1}\ cm^{-1}$)	υ_{cal} (cm^{-1})	*f*
1	30000	3660	31300	0.174
2	29900	4250	31200	0.168
3	30200	6510	31700	0.154
4	29700	5940	31800	0.146
5	--	--	31200	0.084
6	28300	6550	28500	0.158
7	29200	10000	--	--
	30500	12900	31000	0.358
8	29500	3730	29900	0.05
9	29000	7850	30000	0.511
10	29100	6580	30300	0.432
11	29100	8390	29800	0.354
12	29800	6660	30900	0.195
13	30200	14400	29100	0.761
14	30000	14500	29100	0.704
15	29700	16900	29000	0.648
16	30000	17300	29100	0.781
17	29300	19300	28600	0.677
18	30300	10800	30100	0.48
19	30100	23400	29600	0.629
20	29500	16300	29200	0.611
21	29000	13000	--	--
	30300	17400	30800	0.396
22	28500	8070	27800	0.291
23	27900	9370	28400	0.254
24	30200	5750	29200	0.146
25	28900	5350	29300	0.153
26	30100	7230	--	--
	31300	10200	31300	0.334
	32700	10800	31600	0.162
27	26700	6400		
	28000	7200	28700	0.17
	29300	4600		
28	--	--	28100	0.043
29	--	--	28400	0.248

Table 2[10]: Experimental and calculated fluorescence maxima and quantum yield ϕ_F

Table 2	Experimental and Calculated (υ^{exe} and υ^{c10}) fluorescence maxima and quantum yield Φ_F			
Compd	υ_F (cm^{-1})	Φ_F	υ^{exc} (cm^{-1})	υ^{c10} (cm^{-1})
1	26400	0.02	25900	31400
2	26300	0.01	29000	30600
3	26700	0.02	27500	31700
4	25100	0.01	27500	30300
	26500			
5				30700
6	24700	0.033	25400	27300
7	27200	0.052	28800	30800
	29300			
8	25400	0.009	26700	29400
	26400			
9	24400	0.061	26300	
	25600			
10	26100	0.011	23400	
	27300			
11	24000	0.018	23100	
	26000			
12	23300	0.01	27300	29500
	26800			
13	26500	0.009	21500	28300
14	26600	0.008	20800	
15	25500	0.105	24400	
16	25500	0.015		
	26300			
17	25100	0.028	21400	
18	26200	0.005		
19	26400	0.009		
20	25600	0.004	22200	25700
21	27000	0.051	28200	
22	24400	0.04		28100
23	23500	0.046		
24	25600	0.007	24800	29100
	26800			
25	23600	0.022	22100	
	25700			
26	27000	0.026	29100	
27	23600	0.195	26600	28600
	24800			
	26000			
28			25300	27300
29			26100	27200

Results

Several different theoretical approaches were tested to evaluate if the optical spectrum of PICs could accurately be predicted. A number of different methods of geometrical optimization were used, AM1, PM3, and MM+. From X-ray crystallography[1] the symmetry was determined to be C_{2v} micro-symmetry in PICs **11-16c** and **16d** (Figure 3); this orientation occurs while the methyl groups are perpendicular to the plane of the ring system. PICs **1-10a** and **17-18c** have C_1 symmetry (Figure 3). Both AM1 and PM3 were unable to reproduce this C_{2V} micro-symmetry of PICs **11-16c** and **16d**. MM+ was able to more accurately reproduce this symmetry, which agrees with the X-ray crystallographic data. Proper geometric optimization is important since the symmetry will affect the electronic spectra. ZINDO/S was the only method available for the simulation of the electronic spectra.

The actual and theoretical excitation wavelength of PICs are listed in Table 3. The agreement between experimental and calculated excitation energies ranged from an error of 0.02 to 8.8 %, which is the similar to those obtained in a literature study of coumarins. The exiciation maxima was taken to be the stongest oscilator wavelength line just before lowest triplet state. The actual excitation energies were determined in aqueous solution. Figure 5 is a fluorescencent spectrum of PIC **2a**, whose optical properties are representation of the whole family of dyes.

The actual excitation maxima was measured in aqueous solution. The theoretical maxima was compared to the excitation maxima; this comparison is found in Figure 6. The theoretical excitation energies of PIC **14b** had the best agreement with the

experimentally determined values, (error 0.02 %). The poorest agreement obtained was with PIC **15b,** (error 8.8 %).

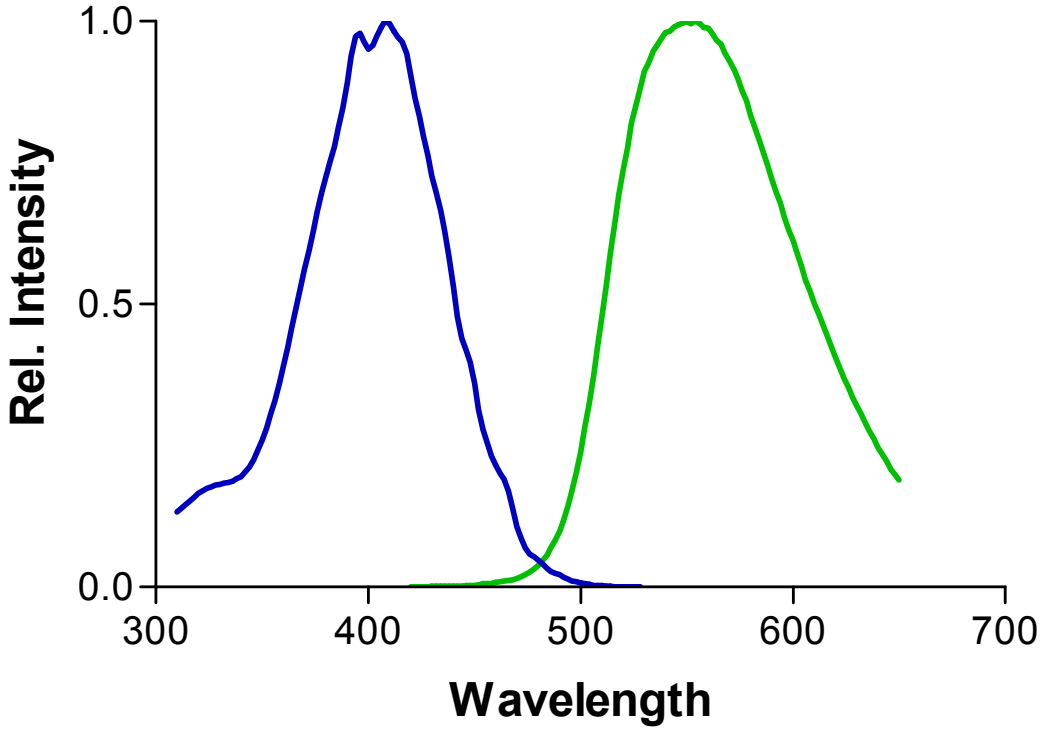


Figure 5: Excitation and emission maxima for PICs 1b

Table 3: Excitation maximum of each dye, their wavelength and strength and the actual excitation maximum

DYE NAME	Actual (nm)	Theory (nm)	Theory (cm^{-1})	Actual (cm^{-1})	Percent Error %
1a	433.7	425	23058.3	23529.4	2.0
2a	434.1	427	23036	21881.8	5.2
3a	436.6	425	22905.9	24449.9	6.3
5a	394.2	413	25367.9	25000	1.5
11b	434.1	425	23259.5	23529.1	1.1
12b	433.1	430	23087.2	23255.8	0.73
13b	443.4	445	22553.8	22471.9	0.36
14b	467.9	468	21371.4	21367.5	0.02
15b	419.17	447	22068.3	24213.07	8.8
17c	477.31	440	20951.7	22222.2	5.7
18c	477.29	440	20950.7	22222.2	5.7
19d	465.32	440	20951.7	22222.2	5.7

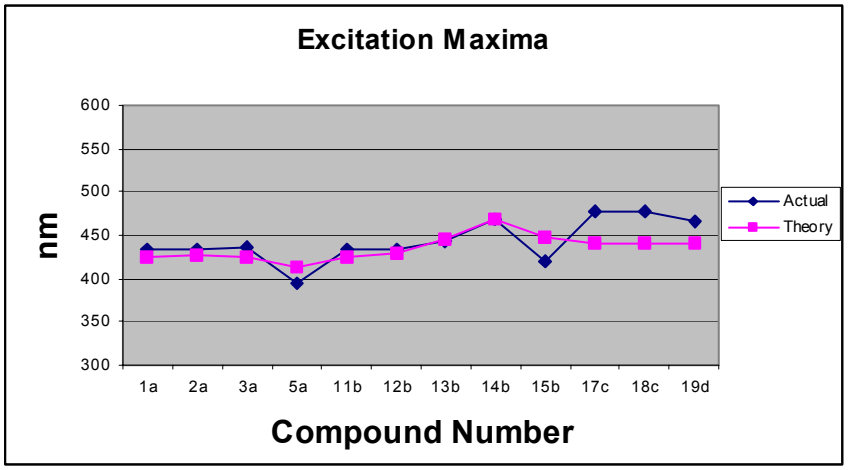


Figure 6: Actual excitation compared to theoretical excitation

The emission energies were computed using the same method as for the excitation energies. The results are summarized in Table 4. As with many organic dyes, the emission spectra of PICs are very broad (Figure 5), especially in aqueous solution. The emission maxima were difficult to simulate due to the large Stoke shift. The emisson maxima were estimated to arise from photo emission from the first triplet state after the excitation. The agreement of theoretical emission and experimentally determination energies ranged from excellent to acceptable (error .18 to 7.8 %, Table 4). The experimentally determined emission energies vs. actual maxima are shown in Figure 7. The best agreement occurred with cation **5a** (error .10 %). PIC **3a**, showed the poorest correlation (error 7.3 %).

Dyes	Actual Emission (nm)	Theoretical Emission (nm)	Theoretical Emission (nm)	Actual Emission (nm)	Percent Error %
1a	494	519	19263.5	20242.9	4.8
2a	505	521	19230.4	19802	2.8
3a	490	529	18903	20408.2	7.3
5a	546	546	18315.1	20449.9	.18
6a	573.1		17449.9		
11b	533	552	18097.1	18761.7	3.5
12b	524	557	17950.7	19084	5.9
13b	571	571	17502.1	18148.8	.18
14b	626	625	15983.8	14814.8	.18
15b	540	540	18512.5	18181.8	.18
17c	550	562	17776.6	22222.2	2.1
18c	550	557	17943.9	22222.2	2.1
19d	550	566	17666.7	22222.2	2.1

Table 4: Theoretical and actual emission maximum

Almost all theoretical emission maxima were at lower energy than actual emission maxima. This was not the case in the study of coumarins. This was likely caused by the way the emission maxima were determined, which was the first triplet state after the excitation maxima. This methodology of using the triplet was used since the computational methodology was unable to correctly evaluate the PICs system because of its unusually large Stoke shift (100 nm). Due to this problem there were no singlets, only triplets, where the expected emission maxima should have been located. So it was theorized that this triplet state did in fact exist in the molecule. The 77 K spectrum of PIC **2a** (Figure 4) shows a shoulder on the emission maxima, which is thought to be a triplet state. The triplet is lower in energy than the expected singlet; this insures that the prediction of emission maxima is at a longer wavelength than the actual maxima in normal fluorescent. The only exception is dye **14b;** this is the only red dye (675 nm) in the family of dyes. Dye **14b**'s stoke's shift is unusually large even for this family of dyes

(200 nm), this astronomically large shift may break down the computational methodology.

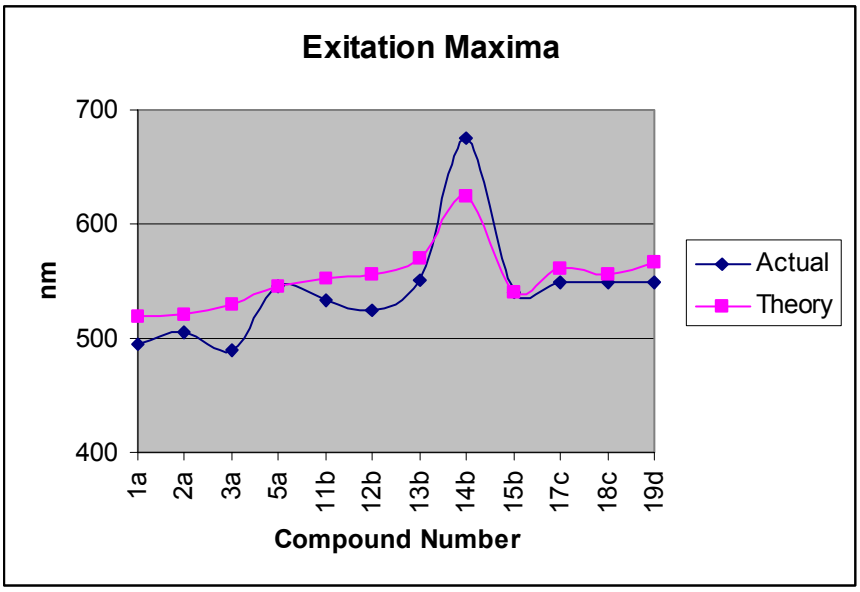


Figure 7: Actual emission maximum compared to actual emission maximum

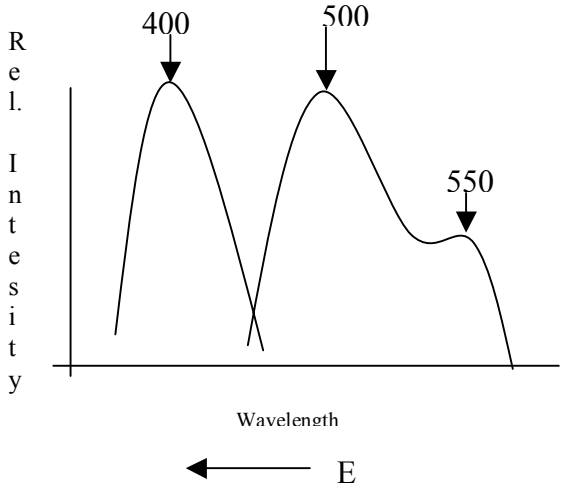


Figure 8: A representation of the shoulder on the 77 K data.

Methods

Chemical structures were drawn with Isis Draw software.[12] The structure was imported into HyperChem 6.0. A log was then initiated for the purpose of recording the results and a sample is contained in Appendix I. Optimization was performed, using MM+. Once an optimization for PICs **11-16b** and **19d** agreed with the known symmetery (C_{2v}), an electronic spectra was obtained. PICs **1-10a** and **16-17c** were optimizated to give known symmetery (C_1), then an electronic spectra was obtained. The parameters for semi-emprical methods were set according to Table 5.

	PICs **1-16**	PICs **17-18**
Total Charge	1	2
Multiplicity	1	1
Spin Pairing	RHF	RHF
State	Lowest	Lowest
Weight factor $\sigma-\sigma$	1.267	1.267
Weight factor $\pi-\pi$	0.585	0.585
Occupied Orbitals	5	5
Unoccupied Orbitals	5	5

Table 5: Parameter for semi-empirical methods

The electronic spectra was calcaluted with the use of the semi-empricial method Zindo/s. The excitation wavelength was determined to be the stongest oscilator line in the spectra just before the lowest triplet state**.** The emisson maxima was determined to arise from photo emission from the first triplet state after the excitation.

This work has been used to identify a potential red fluorescent dye, **6a;** low molecular weight red fluorescent dyes would potentially be useful in imaging cells. The

theoretical data shows that amino groups in the 4 and 4' position of the pyridine ring would result in the longest wavelength emission.

Conclusion

The actual excitation maxima were measured in the aqueous solution. The theoretical maxima and the excitation maxima were compared in Figure 6. The theoretical excitation energies of PIC **14b** had the best agreement with the experimentally determined values, (error 0.02 %). The poorest agreement obtained was with PIC **15b** (error 8.8 %).

The agreement of theoretical emission energies and experimentally determined values obtained ranged from excellent to acceptable (error .18 to 7.8 %, Table 4). The experimentally determined emission energies were compared to the actual emission energies, as shown in Figure 7. The best agreement occurred with compounds **5a** (error .10 %). PIC **3a** showed the poorest correlation (error 7.3 %).

Computational methods were effective in the prediction of both absorption and emission maxima in the PICs system with reasonable accuracy. These computional calculations provide a valuable tool in the rational design of optical properties of dyes in our laboratories with the goal of longer wavelength dyes.

Chapter 2: Synthesis of fluorescent dyes

Introduction

The majority of PICs (Figure 3) prepared in our laboratory are violet excitable green fluorescent dyes (490-540 nm). For many applications, including intracellular studies, longer wavelength dyes (<600 nm) are advantageous. Amino derivatives of fluorescent compounds generally have shown a red shifted fluorescence compared to the parent fluorophore. This lowering of the emission energy is due to the influence of the lone pair of electrons on the amino nitrogen atom on the energy of the entire ring system. The effect of aminating green fluorescent PICs will be explored. PICs 1 were chosen because there seemed to be multiple ways in which amination can be accomplished, and in multiple positions, using straightforward synthetic methodology. An additional motivation for preparing amino substituted PICs is that it offers several routes to the preparation of an isothiocyanate.

A survey of the literature revealed that Newkome[13] and co-workers developed a methodology toward the preparation of a 5-amino-substituted 2-2'bipyridine (Figure 9). The methodology could be applied to the pyridylquinoline ester, **2a,** (Figure 10) giving the amino pyridyl-quinoline ester, **8a, 10a,** (Figure 11). A survey of the literature revealed that Hawthorne[14] and co-workers reported a method of making an amine into an isothiocyanate.

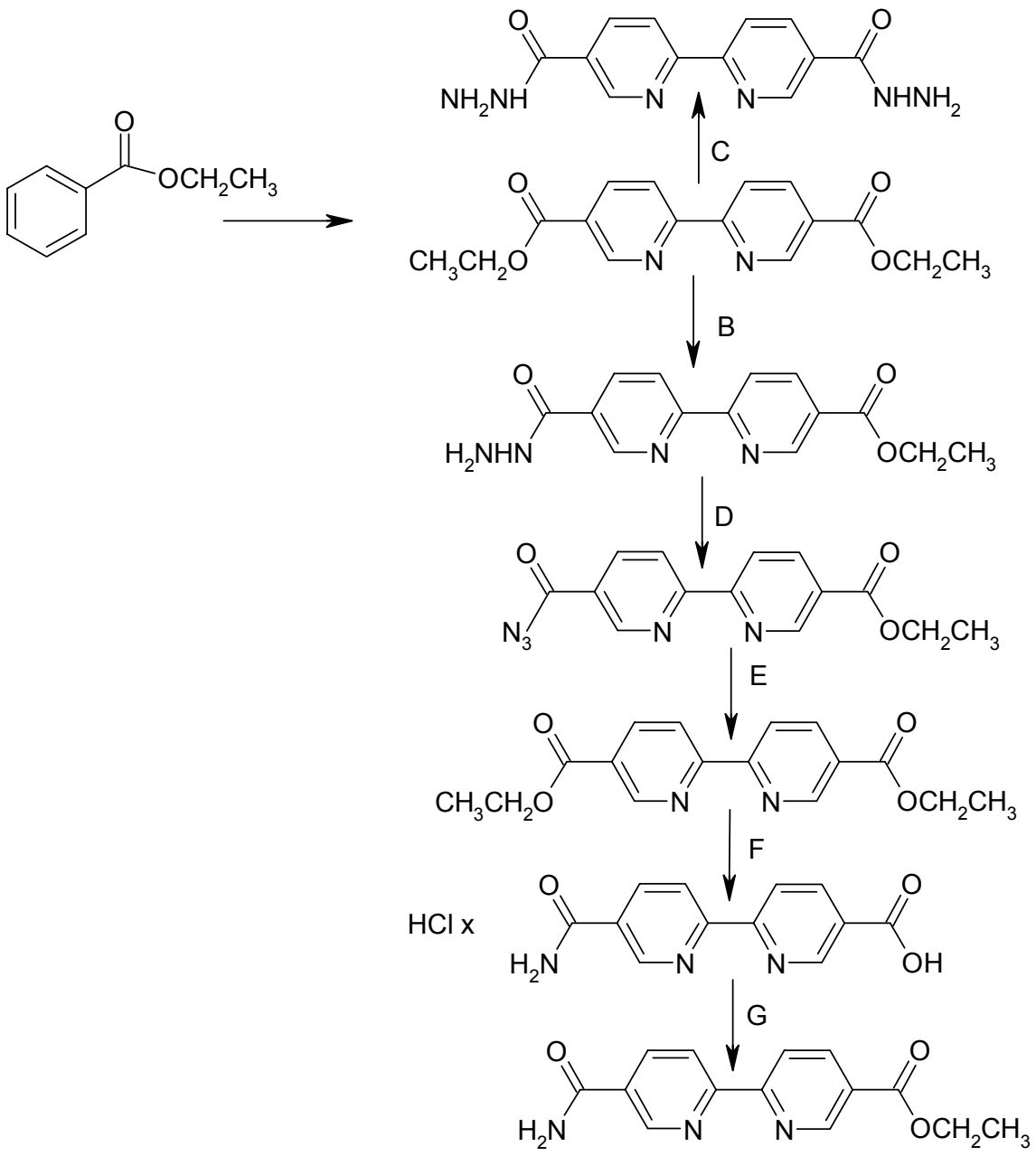


Figure 9: Scheme 1 (a) 10% Pd/C (b) 1.5 equiv of NH_2NH_2, EtOH, toluene, 115 ^{0}C (d) HCl, 0^{0}C, aqueous $NaNO_2$ (e) EtOH, xylene, reflux (f) EtOH, 2.5 M NaOH, 75^{0}C (g) con. H_2SO_4

The ester functionality was synthetically manipulated into an amine in multiple high yield steps (Figure 10), with the key step being a Curtis rearrangement of the azide, **3a,** into the ethyl carbamate, **4a**. This methodology was chosen based on the work of

Newkome[14] in a similar synthesis of a 5-aminosubstituted bipyridine (Figure 9). Newkome showed that a Curtis rearrangement in these related compounds could be extremely effective. Thus, in our preparation of a 4-amino-2-(2-pyridyl)-4-aminoquinoline, **5a,** this methodology was an obvious starting point. In practice the strategy worked extremely well. All of the steps went in good to very good yield, could easily be adopted to large scales, required no chromatographic separations, and produced the target amine in an overall yield of (35%) from 4-carboxymethyl-2-(2-pyridyl)-quinoline.

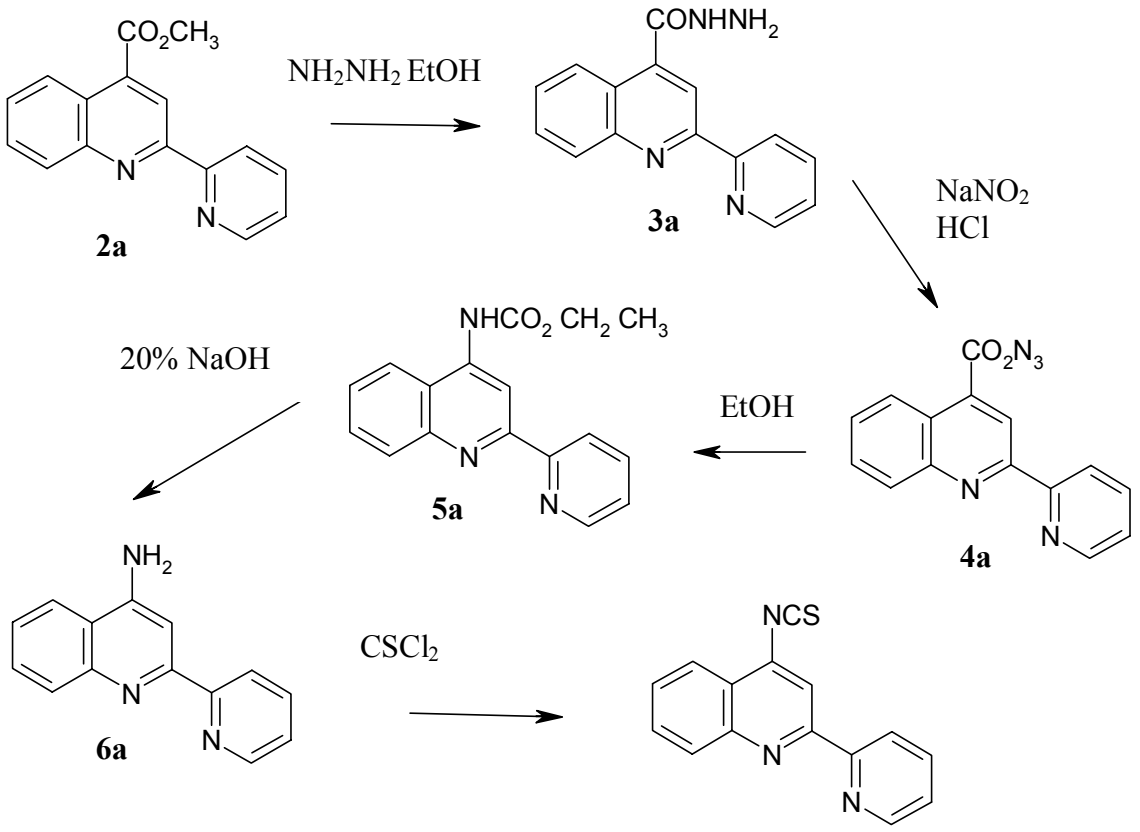


Figure 10: Scheme used to synthesize amino PICs

The second approach toward an amino substituted pyridyl-quinoline derivative was to directly nitrate the ester, **2a,** followed by reduction of the nitro group (Figure 11). Nitration of the ester, **2a,** gave a set of isomers in a 1:1 mixture, that were unable to be separated. Reaction temperature had little or no effect on the ratio of isomers. It was reasoned that the amines might be easier to separate than the corresponding nitro compounds. The nitro mixture was reduced with PtO_2 to the amino compounds. This approach proved to be successful on 4-carboxymethyl-8-amino-2-(2-pyridyl)quinoline, **10a,** because it was soluble in methylene chloride. All of the steps in Figure 11 went in good yield, required no chromatographic separations, and produced the target amine in an overall yield of (41%).

Part of this research was targeted the preparation of a fluorescent isothiocyanate PIC dye. In 1941 Albert Coons developed the fluorescent isothiocyanate immunofluorescene technique.[15] Immunofluorescence involves the attachment of a fluorescent dye to an antibody with the use of an isothiocyanate group. Isothiocyantes are known to be chemically reactive with nucleophillic groups on proteins. The isothiocyanate was to be prepared from the amino coumpound, **5a**. The methodology proposed by Hawthorne[14] was attempted, which utilized thiophosgene. The attempt was unsuccessful in yielding the expected isothiocyanate. The reaction was attempted under nitrogen and in air; neither was effective. Also, other reaction conditions were tried using thiophosgene and were also ineffective.

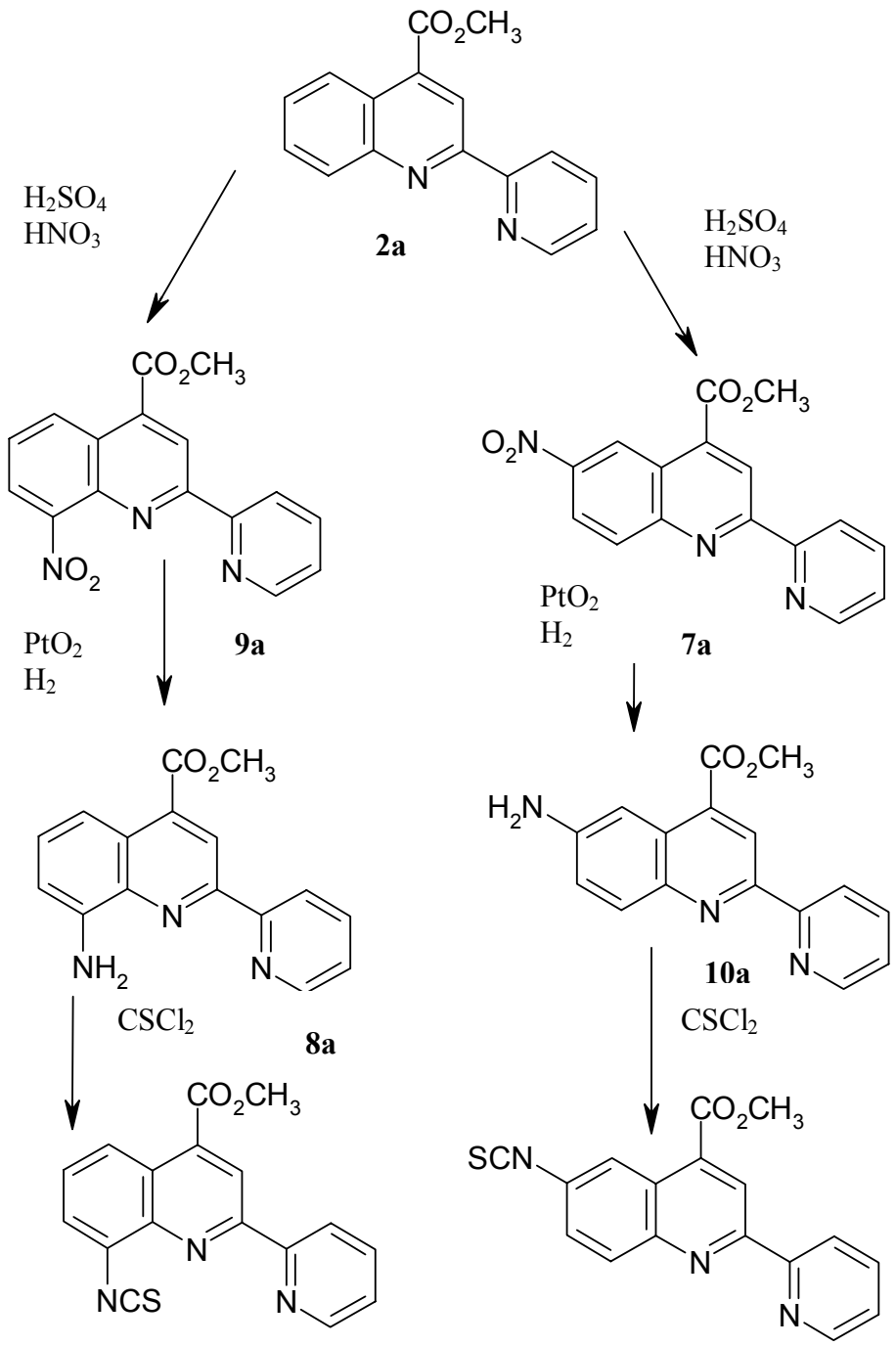


Figure 11: Synthesis of Amino-ester PICs

Experimental

All chemicals were purchased from chemical suppliers and used without further purification, unless noted

4-Carbohydrazido-2-(2-pyridyl)quinoline, **3a**

To a 1 L round bottomed flask was added 10.1g of 4-carboxymethyl-2-(2-pyridyl)-quinoline, **2a** (19.0 mmol) 50 mL of xylene, 30 mL of ethanol, and 30 mL of hydrazine hydrate was added. The stirred solution was refluxed for 20 hours. The flask was cooled to 5 ^{0}C and the solid filtered, and allowed to air dry, giving 6.5 g of **3a** as a bright white solid (65%) mp. 221-223 ^{o}C. ^{1}H NMR (DMSO-d_6) (Figure 12) COSY (Figure 13) δ = 10.0 (s, 1 H, NH); 8.78 (d, 1 H, J = 4.5, H-d); 8.63 (d, 1 H, J = 7.5, H-a); 8.56 (s, 1 H, H-o); 8.18 (d, 1 H, J = 8.5, H-4); 8.21 (d, 1 H, J= 8.5, H-1); 8.04 (ddd, 1 H, J = 2,1.5,8,8, H-b); 7.86 (ddd, 1 H, J = 1,2,1.5,7,8,7.5, H-3); 7.70 (ddd, 1H, J = 1,2,1.5,7,8,7.5, H-2); 7.56 (ddd, 1 H, J = 1.5,1,5,7.5, H-c) 4.75 (s, 2 H, NH_2) ^{13}C NMR (DMSO-d_6) δ = 165.99, 154.98, 154.57, 149.37, 147.54, 142.08, 137.51, 130.27, 129.61, 127.65, 125.46, 124.93, 124.50, 121.07, 116.47 IR NH_2 (3425 cm^{-1})

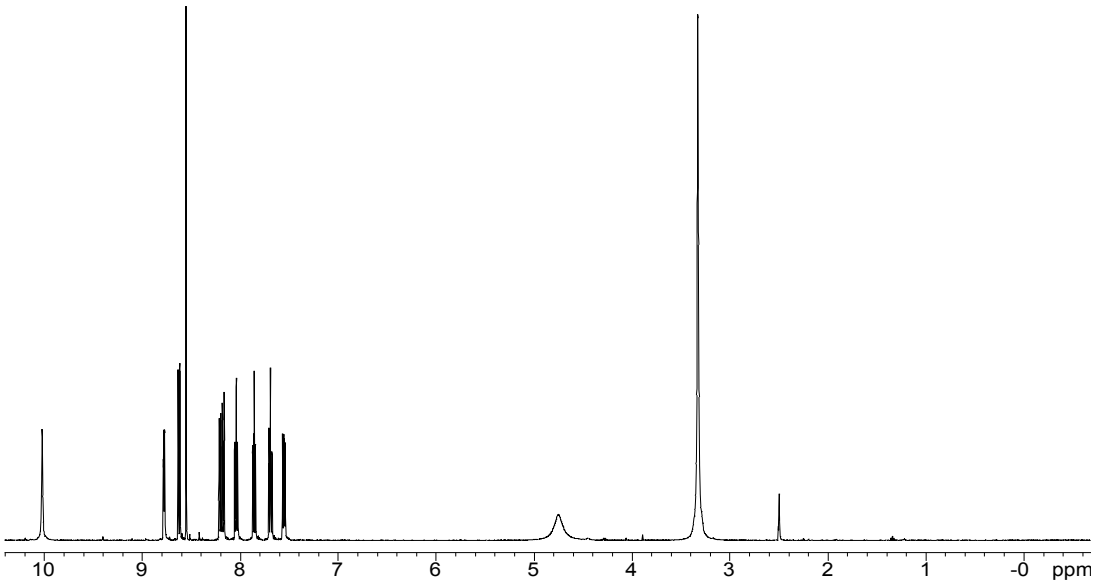


Figure 12: ^{1}H 500 MHz NMR of 4-carbohydrazido-2-(2-pyridyl)quinoline.

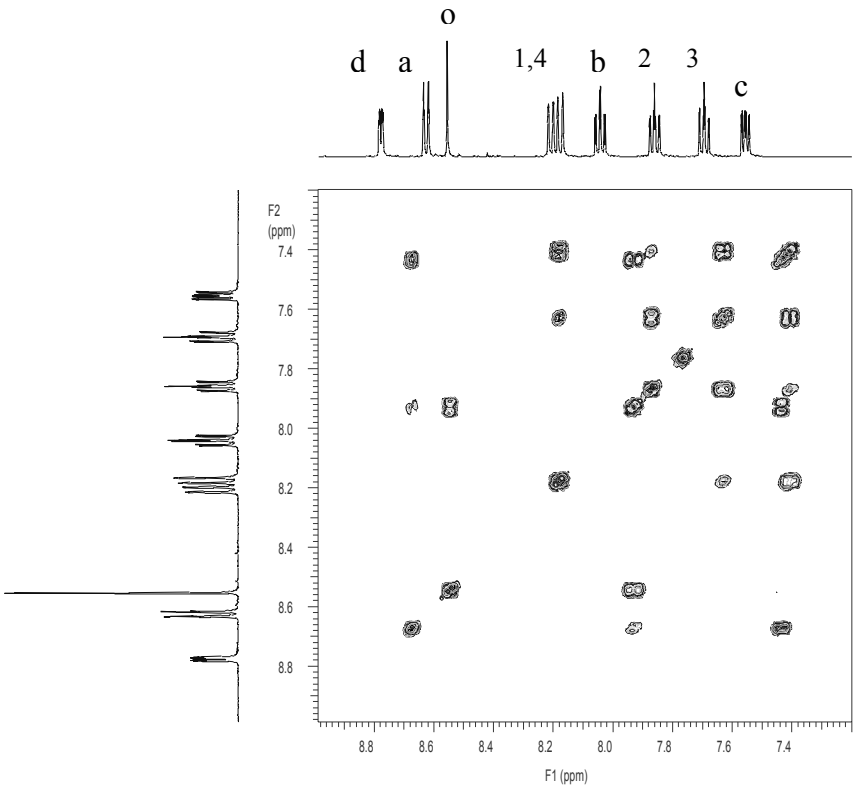


Figure 13: $^{1}H^{1}H$ COSY 500 MHz NMR of 4-carbohydrazido-2-(2-pyridyl)quinoline

4-Carbazido-2-(2-pyridyl)quinoline, **4a**

A two neck 1 L round bottomed flask was fitted with a magnetic stirrer, ~~a~~ thermometer, and a pressure equalized dropping funnel. The flask was charged with 90 mL of con HCl and 6.4 g (10 mmol) of 4-carbhydrazido-2-(2-pyridyl)quinoline, **3a**. The solution was cooled to 0 ^{o}C in an ice bath. The funnel was charged with a solution of 30 g (432 mmol) of sodium nitrite in 200 mL of water. With stirring, the solution of sodium nitrite was added to the flask at a rate such that the temperature remained below 5 ^{o}C. Too high a rate of addition causes foaming to occur, particularly if the temperature rises too swiftly. During the addition the color of the solution changes from a bright yellow to a burnt orange. Following the addition of the sodium nitrite solution, stirring was continued for an additional 15 minutes at 0 ^{o}C, and 1 hour at room temperature. At this

point there the solution had a pale yellow coloration with a thick white precipitate. The addition funnel was charged with a solution of 10% NaOH, and 100 g of ice was added to the flask. The reaction mixture made basic. Small portions of ice were added to the flask so as to maintain the temperature below 2 ^{o}C throughout the neutralization. The pale white precipitate **3a** was collected by suction filtration, washed liberally with water (3 X 100 mL, placed in a 100 mL round bottomed flask and dried under vacuum at 20 ^{o}C. giving **4a** as a white solid, which was found to be thermally stable up to 95 ^{o}C (5.5 g, approximately 68%). It was purified, but used immediately in the preparation of **5a**.

4-Ethylcarbamato-2-(2-pyridyl)quinoline, **5a**

To a 100 mL round bottomed flask, was added 4-carbazido-(2-pyridyl)quinoline, **4a,** 40 mL of xylene and 30 mL of absolute ethanol. The mixture was refluxed for 6 hours. The temperature was closely controlled to avoid severe frothing when the reaction mixture was refluxing. The mixture was then cooled to room temperature, and the volume reduced on a rotory evaporator until a thick white precipitate appeared. At this point the mixture was cooled to 5 ^{o}C and filtered, giving the carbamate ester, **5a**, as a white solid mp 171-173 (65 % yield). ^{1}H NMR (DMSO-d_6) (Figure 14) COSY (Figure 15) δ = 8.78 (d, 1 H, J = 4.5, H-d); 8.62 (d, 1 H, J = 8, H-a); 8.55 (s, 1 H, H-o); 8.21 (d, 1 H, J = 8, H-4); 8.18 (d, 1 H, J = 8.5, H-1); 8.04 (ddd, 1 H, J = 1.5,2,8,8, H-b); 7.86 (ddd, 1 H, J = 1.5,2.5,1,7.5,8, H-3); 7.70 (ddd, 1 H, J = 1.5,2.5,1,7.5,8,7.5, H-c); 7.55 (ddd, 1 H, J= 1,1.5,5,5,7.5) 4.34 (t, J = 5, CH_3) 3.42 (q, J = CH_2) δ =165.99, 154.98, 154.56, 149.36, 147.54, 142.08, 137.49, 130.25, 129.60, 127.64, 125.45, 124.92, 124.49,

121.06, 116.46, 55.98, 18.49 IR NH (3462 cm^{-1})

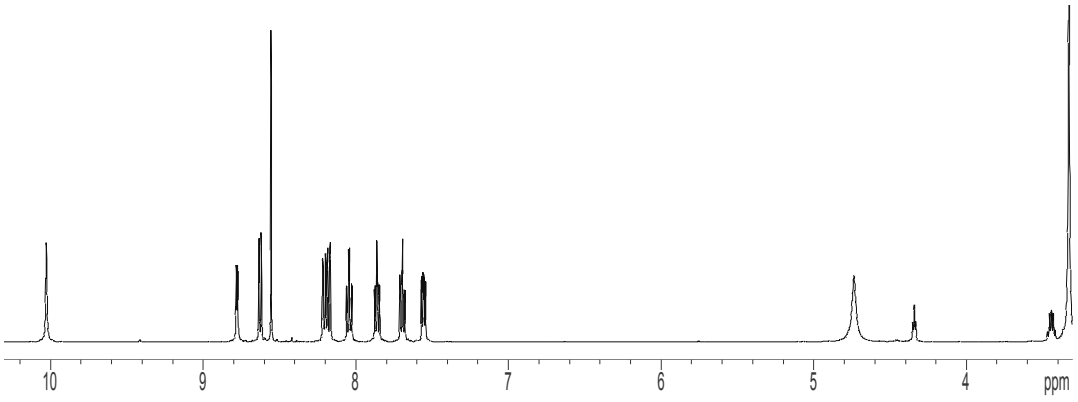


Figure 14: ^{1}H 500 MHz NMR of 4-ethylcarbamato-2-(2-pyridyl)quinoline.

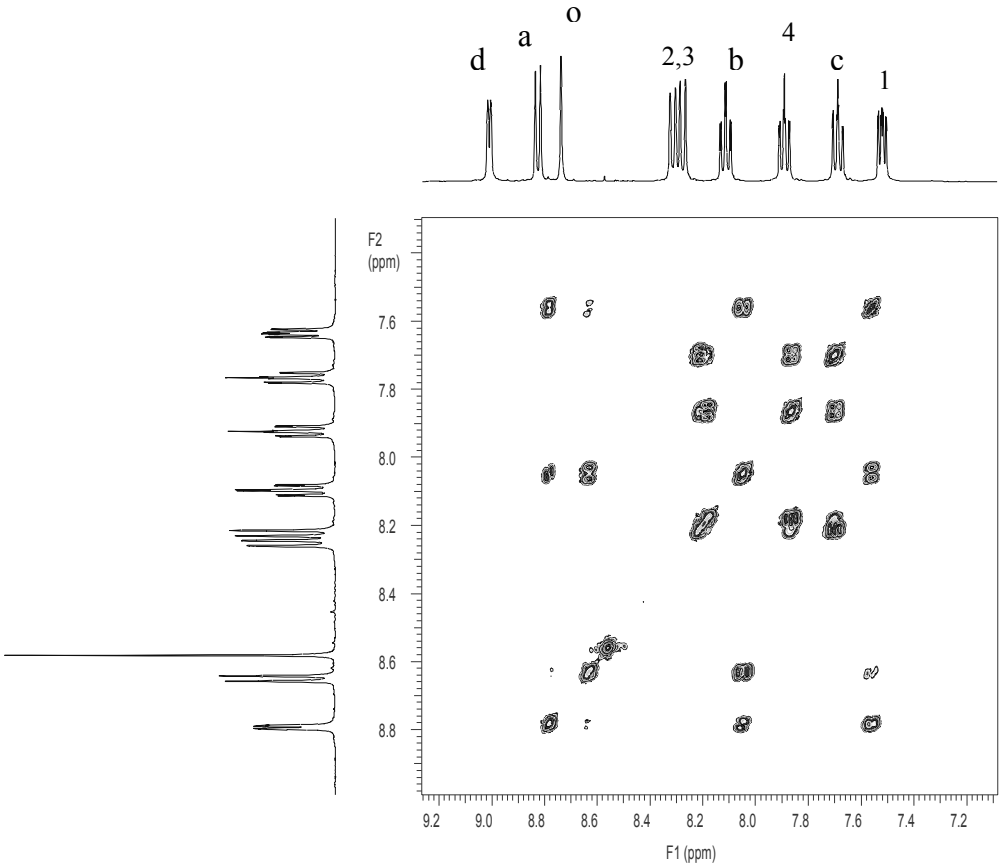


Figure 15: $^{1}H^{1}H$ COSY 500 MHz NMR of 4-ethylcarbamato-2-(2-pyridyl)quinoline

4-Amino-2-(2-pyridyl)quinoline, **6a**

A stirred solution of 4-ethylcarbamate-2-(2-pyridyl)-quinoline, **5a,** (5.0 g) in a mixture of ethanol (50 mL) and 2.5 N aqueous NaOH (50 mL) was heated at 75°C for 14 hours. The ethanol was concentrated in a rotary evaporator, and the result white precipitate, **6a,** (67%) was vacuum filtered, washed with cold water, and air-dried. ^{1}H NMR (DMSO-d_6) (Figure 16) COSY (Figure 17) δ = 8.68 (d, 1 H, J = 5, H-d); 8.54 (d, 1 H, J = 8, H-a); 8.18 (d, 1 H, J = 8.5, H-4); 7.67 (dd, 1 H, J = 8,7,7.5, H-2); 7.93 (ddd, 1 H, J = 1.5,7.5,8,7.5, H-b); 7.87 (d, 1 H, J = 8.5, H-1); 7.77 (s, 1 H, H-0); 7.4 (m, 1 H); 7.4 (m, 1 H) 6.9 (s, 2 H, NH_2) δ = 156.26, 155.35, 148.82, 148.53, 136.81, 129.26, 129.19, 123.92, 123.85, 122.25, 120.79, 118.53, 99.21, 30.61. IR NH_2 (3375 cm^{-1})

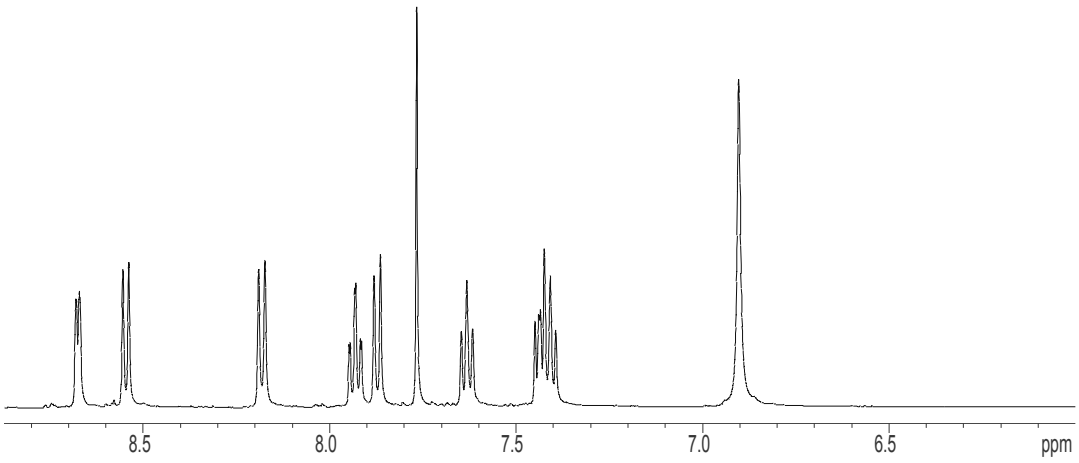


Figure 16: ^{1}H 500 MHz NMR of 4-amino-2-(2-pyridyl)quinoline

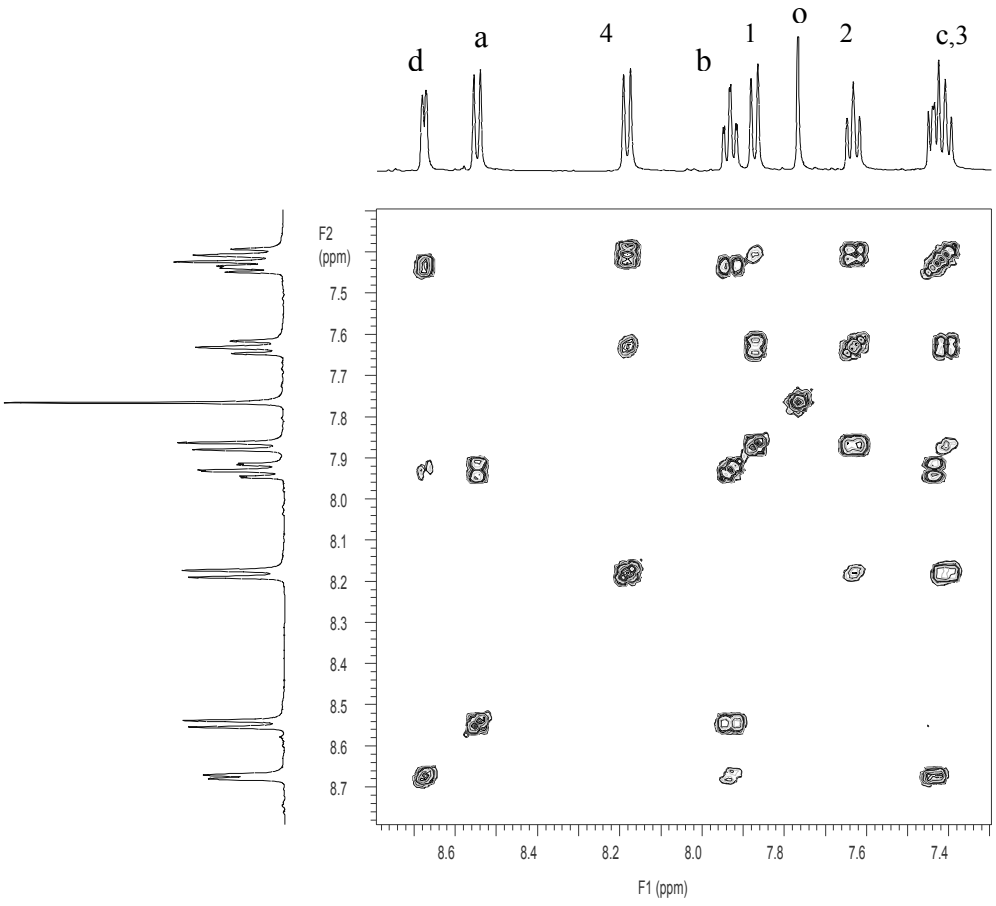


Figure 17: $^{1}H^{1}H$ COSY 500 MHz NMR 4-amino-2-(2-pyridyl)quinoline

Mixture of 4-Carboxymethyl-6-nitro-2-(2-pyridyl)quinoline,**7a**, and 4-Carboxymethyl-8-nitro-2-(2-pyridyl)quinoline, **9a**

A two neck 250 mL round bottomed flask was fitted with a magnetic stirrer a thermometer, and a pressure equalized dropping funnel, to the flask was added 25 mL of con sulfuric acid and 5.0 g (4.0 mmol), of 4-carboxymethyl-2-(2-pyridyl)quinolinie, **2a**. The solution was cooled to 0 ^{0}C in an ice bath. The addition funnel was charged with a solution of 25 mL of con nitric add. With stirring, the nitric acid was added to the flask at a rate such that the temperature remained below 5 ^{0}C. Following the addition, stirring was continued for an additional 15 minutes at 0 ^{0}C, and 30 minutes at 50 ^{0}C. This mixture

poured over 100 g of ice and neutralized with 10% NaOH. The precipitate was collected by filtration washed liberally with water and allowed to dry, giving the nitro compounds, **7a, 9a,** as a pale yellow solid (5.2 g, 86%), which was found to be a mixture of isomers. The isomers were 4-Carboxymethyl-6-nitro-2-(2-pyridyl)quinoline, **7a** and 4-Carboxymethyl-8-nitro-2-(2-pyridyl)quinoline, **9a,** (Figure 18). The COSY spectra are in Figure 19. 4-Carboxymethyl-8-nitro-2-(2-pyridyl)quinoline, **7a,** δ = 9.062 (s, 1 H, J = 5, H-o), 9.046 (d, 1 H, J = 1, H-9), 8.743 (ddd, 1 H, J = .5, 1, .5, 1.75, 5, H-k), 8.638 (dd, 1 H, J = 1, 1.5, 1.25, H-i), 8.050 (dd, 1 H, J = 1,7.5, H-11), 7.883 (m, 1 H), 7.696 (dd, 1 H, J = 7.5, 9), 7.409 (m, 1 H) IR NO_2 (1529, 1381 cm^{-1}) 4-Carboxymethyl-8-nitro-2-(2-pyridyl)quinoline, **9a,** ^{1}H NMR δ = 9.250 (s, 1 H, H-o), 8.771 (ddd, 1 H, J = .5, 1, .75, 1.5, 5, H-d), 8.653(dd, 1 H, J = 1, 1.5, 1.25, H-b), 8.540 (dd, 1 H, J = 1.5, 8.5, H-3), 8.172 (dd, 1 H, J = 1.5, 1, 7.75, H-1), 7.904 (m, 1 H), 7.815 (dd, 1 H, J = 7.5, 8.5, H-2), 7.424 (m, 1 H)

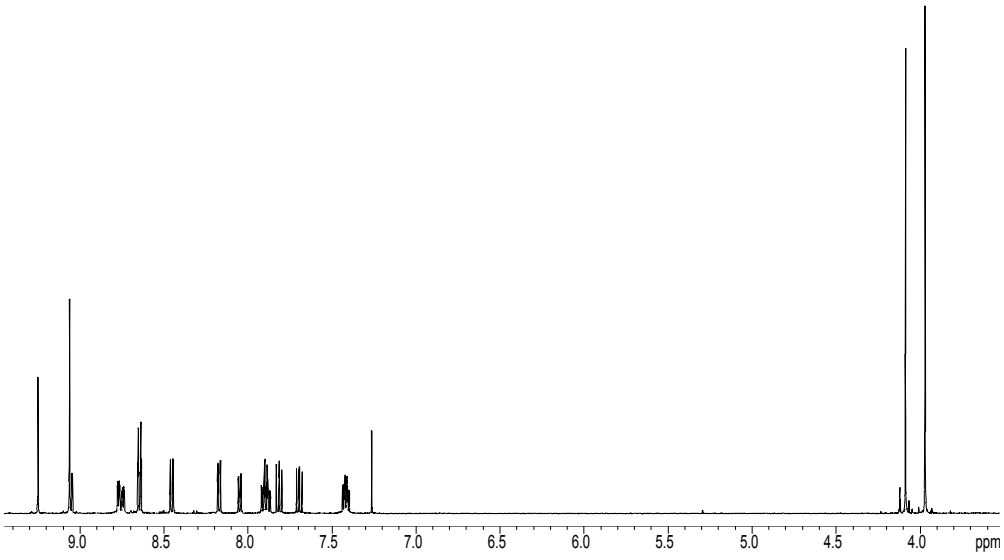


Figure 18: ^{1}H 500 MHz NMR of 4-carboxymethyl-8-nitro-2-(2-pyridyl)quinoline and 4-carboxymethyl-6-nitro-2-(2-pyridyl)quinoline

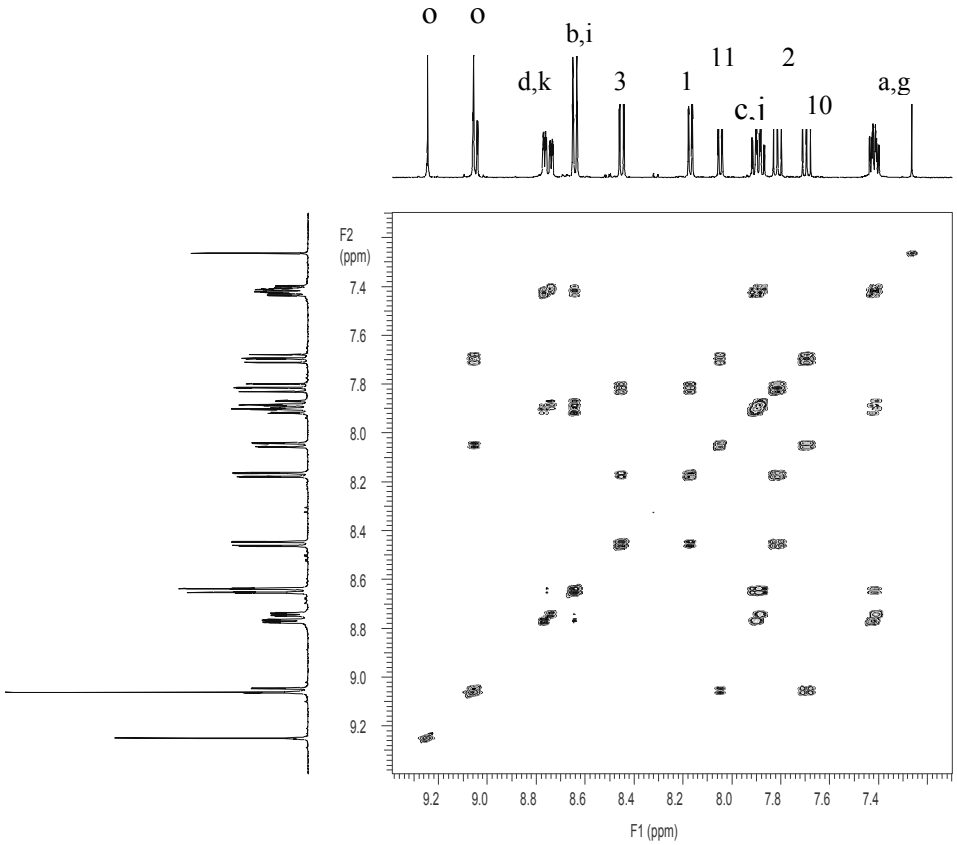


Figure 19: $^{1}H^{1}H$ COSY 500 MHz NMR of 4-carboxymethyl-8-nitro-2-(2-pyridyl)quinoline and 4-carboxymethyl-6-nitro-2-(2-pyridyl)quinoline

Mixture of 4-Carboxymethyl-6-amino-2-(2-pyridyl)quinoline,**8a**, and 4-Carboxymethyl-8-amino-2-(2-pyridyl)quinoline, **10a**,

In a Parr bomb, 5.0 g (4.0 mmol) of 4-CarboxymethyI-8-nitro-2-(2-pyridyl)quinoline, **7a,** and 4-Carboxymethyl-6-nitro-2-(2-pyridyl)quinoline, **9a,** and 0.40 g of platinum oxide were added into methanol. Hydrogen gas was added at a pressure of 2 atm. The reaction was stirred for 2 hours at room temperature. The solution was filtered and the methanol was evaporated. The compound 4-carboxymethyl-8-amino-2-(2-pyridyl)-quinoline, **10a,** was soluble in methylene chloride. Compound 4-carboxymethyl-6-amino-2-(2-pyridyl)-quinoline, **8a**, was soluble in DMSO. IR NH_2 (3395 cm^{-1}) 4-Carboxymethyl-amino-2-2-pyridyl-quinoline, **8a,** (Figure 20) COSY (Figure 21) δ = 9.02 (s, 1 H, H-o), 8.59 (ddd, 1H, H-d), 8.740 (m, 1 H, H-a) 8.038 (dd, 1

H, H-3), 7.846 (ddd, 1 H, H-c), 7.432 (dd, 1 H, H-2), 7.350 (ddd, 1 H, H-b). 6.978 (dd, 1 H, H-1), 5.25 (s, 2 H, NH_2) ^{13}C) δ = 155.934, 149.504, 137.250, 130.613, 130.042, 128.353, 125.781, 52.827 125.325, 124.580, 121.922, 120.536

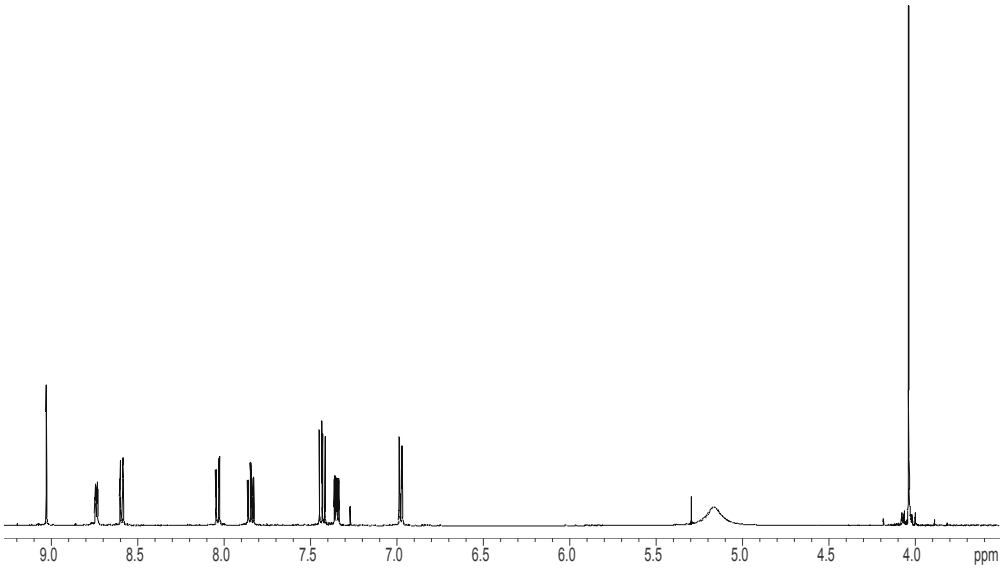


Figure 20: ^{1}H 500 MHz NMR of 4-carboxymethyl-8-amino-2-(2-pyridyl)quinoline

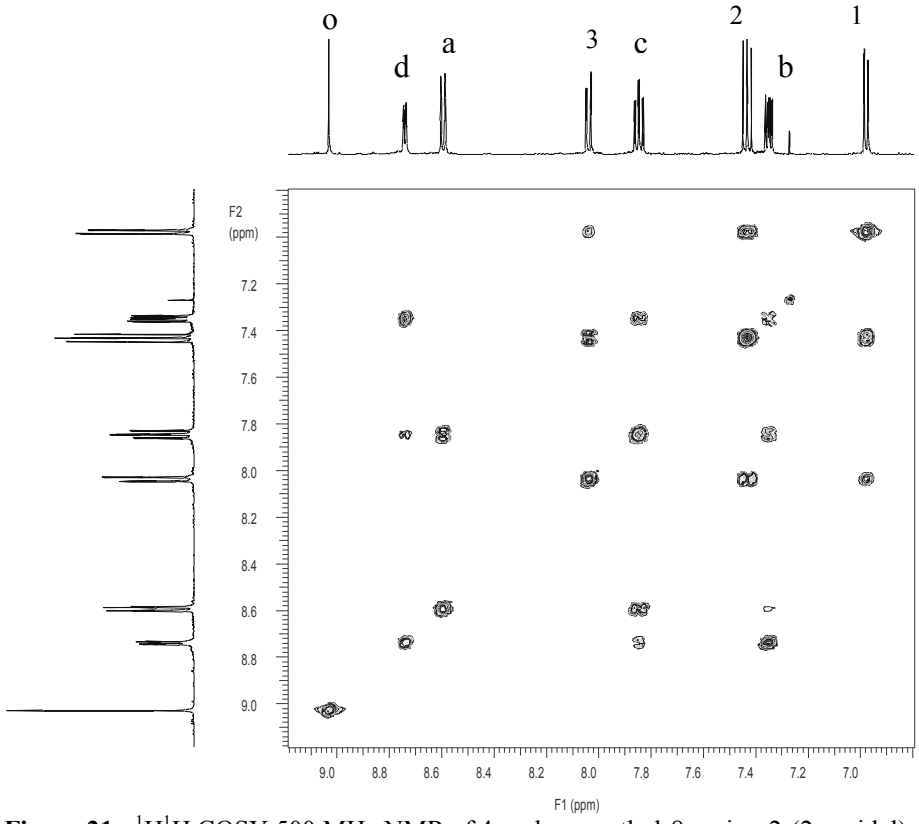
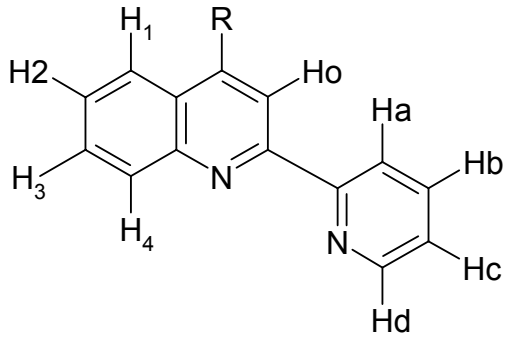


Figure 21: $^{1}H^{1}H$ COSY 500 MHz NMR of 4-carboxymethyl-8-amino-2-(2-pyridyl)quinoline

Results and Discussion

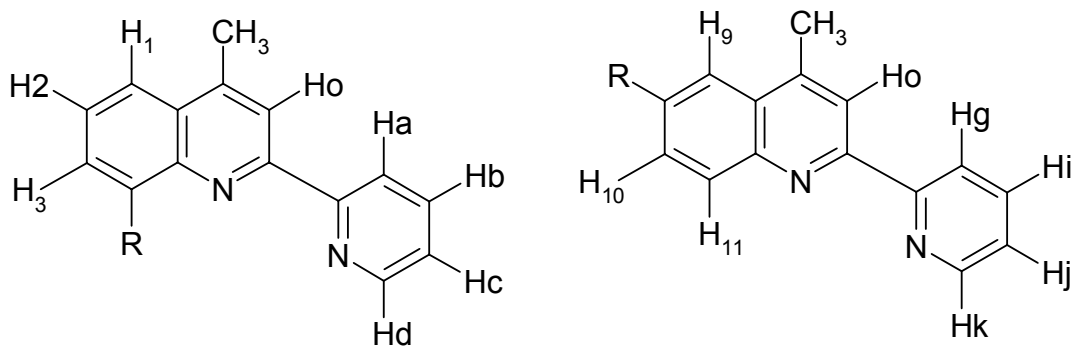
The methodology developed by Newkome[14] (Figure 9) proved to be effective on our system (Figure 10). All steps in the preparation of **5a** (Figure 9) gave a satisfactory yield (approximately 70%) and good purity. The compound 4-amino-2-(2-pyridyl)quinoline, **5a**, was characterized by assigning all of the hydrogens (Figure 22) with the use of COSY NMR and the splitting patterns. COSY NMR is a two dimensional technique which monitors the hydrogen-hydrogen interactions. Hydrogens on the pyridine rings were assigned letters and the hydrogens on the quinoline ring were assigned numbers (Figure 22). COSY NMR allows for the determination of what hydrogens are side by side and the placement of weakly coupled hydrogens. Chemical shift knowledge is also needed in the assignment of hydrogens. In **5a** (Figure 17), the hydrogen most down field is hydrogen d, which would strongly interact with hydrogen c and weakly interact with hydrogen b. A COSY NMR spectra is interpreted the following way, if hydrogen d is followed straight down and hydrogen c is noted by a large dot (strong coupling) and hydrogen b is noted by a relatively small dot (weak coupling). Also in the spectrum there is a dot where hydrogen d interacts with itself. All the hydrogens interacting with themselves forms a diagonal line in all COSY spectrum from the bottom left corner and goes to the top right corner. Once all the hydrogens associated with the pyridine ring had been assigned this left only the hydrogens associated with the quinoline ring. The hydrogens associated with the quinloine ring were assigned the same manner as the pyridine ring. This is the way the hydrogens in all compounds were assigned. FT-IR was used to confirm the presence of an amine group 3375cm^{-1}.



1a = CO_2CH_3 **2a** = $CONHNH_2$ **4a** = $NCO_2CH_2CH_3$ **5a** = NH_2

Figure 22: Base structures for assignment by 1H COSY

The combination of ^{13}C, ^{1}H, and COSY NMR were used to confirm the nitration of 4-carboxymethyl-2-(2-pyridyl)quinoline yielded a set of isomers. The isomers were 4-carboxymethyl-8-nitro-2-(2-pyridyl)quinoline, **9a**, and 4-carboxymethyl-6-nitro-2-(2-pyridyl)quinoline, **7a.** Using COSY and splitting patterns all hydrogens were assigned on the pyridyl-quinoloine in the same manner as **5a** (Figure 23). The reduction of the amine with hydrogen yielded the expected isomeric amines. The 4-carboxymethyl-8-amino-2-(2-pyridyl) quinoline, **10a**, was soluble in dichloromethane therfoe it was possible to separate the isomers. The 4-carboxymethyl-6-amino-2-(2-pyridyl)quinoline, **8a**, was not been purified, due to the poor solubility. The nitro compounds were non-fluorescent. Both amino compounds were fluorescent. As expected, the amino group in both cases did affect the fluorescence spectra causing a red shift of 50 nm.



7 = NO_2
8 = NH_2

Figure 23: Base Structures of the assignment of the isomer mixtures

Conclusion

The presences of amino groups on a known fluorescent ring system are well established to markedly affect the optical properties of the dye. Methodology has been developed for the amination of different position on the 2-(2-pyridyl)-carboxyquinoline ring system(4-Carboxymethyl-6-amino-2-(2-pyridyl)quinoline, **8a**, and 4-Carboxymethyl-8-amino-2-(2-pyridyl)quinoline) **11a**, leading to the testing of three new amino substituted fluorescent dyes. Another methodology was effect in the synthesis of 4-amino-2-(2-pyridyl)quinoline, **6a.** This is also the precursor to 4-isothiocyanato-amino-2-(2-pyridyl)quinoline, which can be used as a cellular tag for proteins.

Chapter 3: Confocal Imaging

Introduction

Conventional microscopes are capable of creating images with a depth of field of 2-3 μm. Due to the limited resolving power of most optical microscopes, it is possible to have several objects in the same depth of field producing superimposition of those objects. Superimposition causes a loss of structural details. Superposition of objects causes halos to form around objects of interest. This is especially prominent in fluorescent microscopes. In contrast, confocal microscopes create optical sections, which are 0.5 μm or less thick. This allows for rejection of light out of the focal plane, which minimizes the obscuring of image details[16] (Figure 24). Confocal microscopes have become an increasingly essential analytical tool for the study of the structure and physiology of living cells.

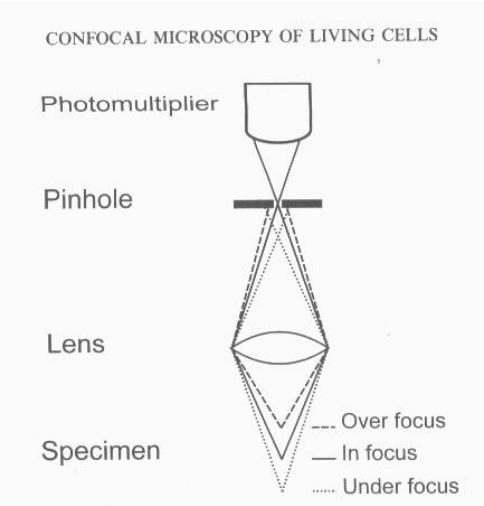


Figure 24: How a Confocal Microscope works

The use of small fluorescent molecules as molecular probes has gained acceptance as one of the most important general methods of obtaining information on cellular structure and function. The pattern of their fluorescence throughout the cell is captured as an image representing a 0.5 μm thick slice of the cell. The combination of many such slices results in a three dimensional image of the cell.

A problem with fluorescent probes is to have the right dye localize in the portion of the cell that is of interest to the observer. Other problems associated with confocal microscopy are photo bleaching and degradation of cells. Photo bleaching is the irreversible destruction of a fluorophore, which decreases the intensity of the dye inside the cell. All dyes have different rates of photo bleaching. With live cells the laser power is very likely to damage and/or kill the cell. Even with all these problems there are hundreds of fluorescent dyes listed in catalogues. Dyes are listed according to their application area (ion-sensitive, membrane potential, etc.) and the organelle in which the dye localize, also included with each dye is a list of literature references.[17] Dyes are subdivided into dyes that stain live cells and those that stain fixed cells. Microinjection is needed when dyes are too large or cannot cross the plasma membrane. Since, there are several ways to classify and subdivided dyes, even if a dyes for that organelle exist for fixed cell, there is still a need for a dyes that stain the same organelle in a living cell.

In our laboratory, a new class of fluorescent dyes (PICs) are made using an exceedingly flexible methodology. This flexibility allows for this family of dyes to be used to study the relationships between dye structure and imaging properties inside cells.

During the course of research, several dyes were studied and their ability to cross the cellular plasma membrane was evaluated. If the dyes did cross the cellular membrane their affinity and/or selectivity properties toward specific organelles needed to be determined.

Cells and their organelles can be selectively labeled. This labeling is based on empirical testing of hundreds of different cells. Several dyes can be used to label specific organelles of living cells. The probes can be excited with multi-line lasers so the probes may be imaged simultaneously.

Confocal microscopes have optical filters that pass only selective wavelengths. When using a confocal microscope it is necessary to understand filters to process and acquire the data correctly. An excitation filter must allow passage of suitable wavelengths of light to allow excitation of the dye, yet eliminate other extraneous light. Emission filters are used to filter light exiting the sample. There are several different types of emission filters (Figure 25). They are divided according to the wavelengths of light they transmit. Long pass filters allow all wavelengths longer than their rated wavelength. A band pass filter is made by a combination of a long and a short pass filter, which allows for a narrow spectral window. The band pass width of the filter will depend on the spectral widths of the individual filters used. Being able to purchase different filters allows customization of the filters to the dyes studied in multi-channel experiments.

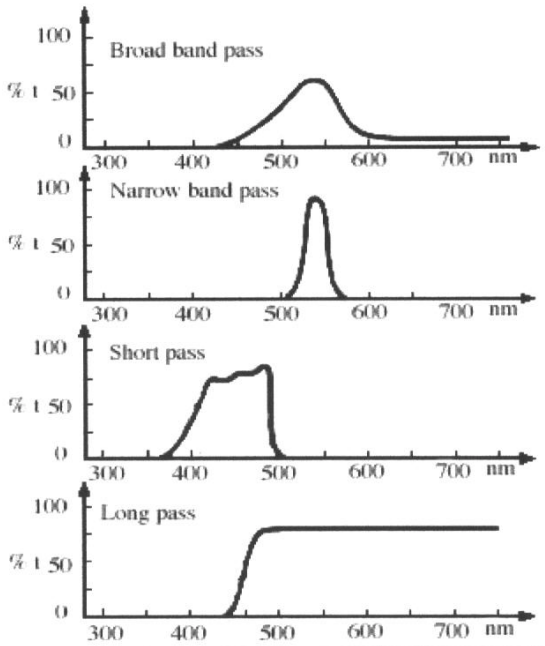


Figure 25: Optical filters for the Confocal Microscope

The utilization of fluorescent probes will certainly require the synthesis of more selective, and more intense fluorescent probes. When new dyes are developed their staining characteristics must be determined. This can be accomplished through multi-channel fluorescent (co-localization) confocal microscopy studies, which are aided by the use of different filters. Confocal microscopes have the ability to use three different filters, simultaneously. Each filter can pass a different spectral window. This allows for known and unknown dyes simultaneously detected in a cell to be monitored through different instrumental channels (filters).

For purpose of comparison Rhodamine 123 (Figure 26) and rhodamine-dextran stain mitochondria and lysosmes, respectively.[18] This staining was accomplished through incubation of cells with 0.1-1 μm of rhodmaine (SP) 123 for 15-30 minutes at the normal growing temperature (37.7˚C). Rhodamine-dextran was incubated at 1 μg/ml overnight.

Mitochondria therefore emitted a green fluorescent color and lysosmes emitted a red fluorescent color.[19]

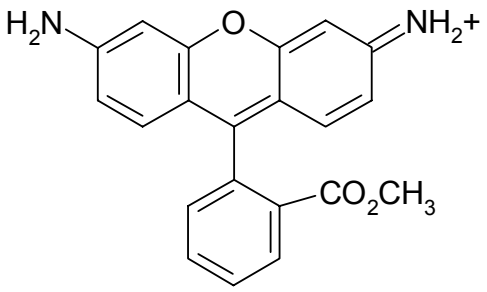


Figure 26: Rhodamine 123

Another example of co-localization is the use of ER-Tracker Blue white DPX®[18] (Figure 27) and MitoTracker Red CM-H_2Xros®[21] (Figure 28), which stain endoplasmic reticulum and mitochondria, respectively. The ER-Tracker Blue White DPX is a green fluorescent dye and MitoTracker Red CM-H_2Xros is red fluorescent dye. The image (Figure 29) was acquired using a fluorescent microscope using a triple-bandpass filter set appropriately for DAPI, Fluorescein, and Texas Red dyes.[21]

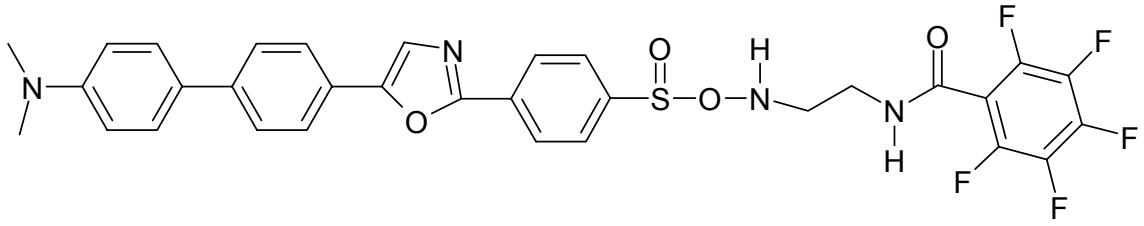


Figure 27: ER-Tracker Blue white DPX

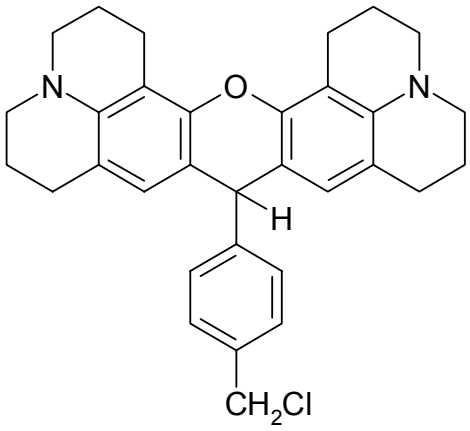


Figure 28: MitoTracker Red CM-H_2Xros

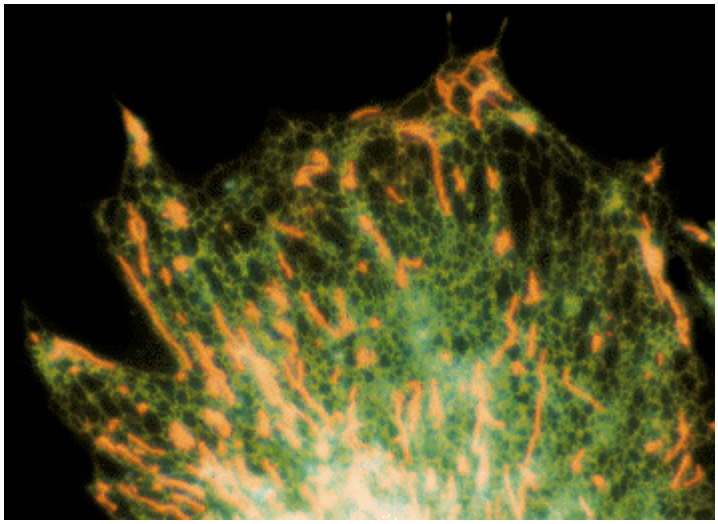


Figure 29[18]**:** Live bovine pulmonary artery endothelial cells stained with ER-Tracker Blue-White DPX and MitoTracker Red CM-H_2XRos. The endoplasmic reticulum appears green and the mitochondria appear orange.

Many other organelles have specific fluorophores designed for them.[17] For example: BODIPY-ceramide derivatives (Figure 30)[20] and NBD-ceramide (Figure 31) are stains specific to the Golgi. Carbocyanine (Figure 32) dyes stain the endroplasmic reticulum,[21] dihydrorhodamine (Figure 33) localize in the mitochondria, and the cytoskeleton can be stained selectively stained using Alexa Fluor 488 (Figure 34).

Phalloidin, DAPI (Figure 35) are used for staining DNA and RNA. Table 6 contains a list of potential applications for certain targeted sub cellular structure.

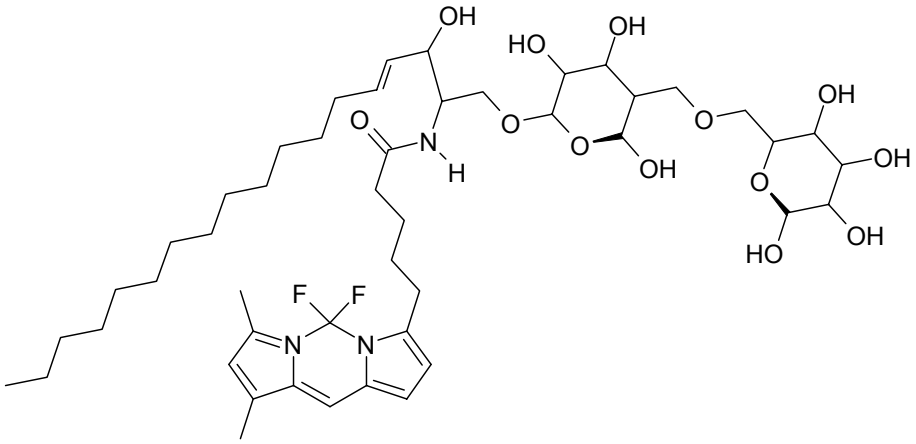


Figure 30: BODIPY-ceramide derivatives

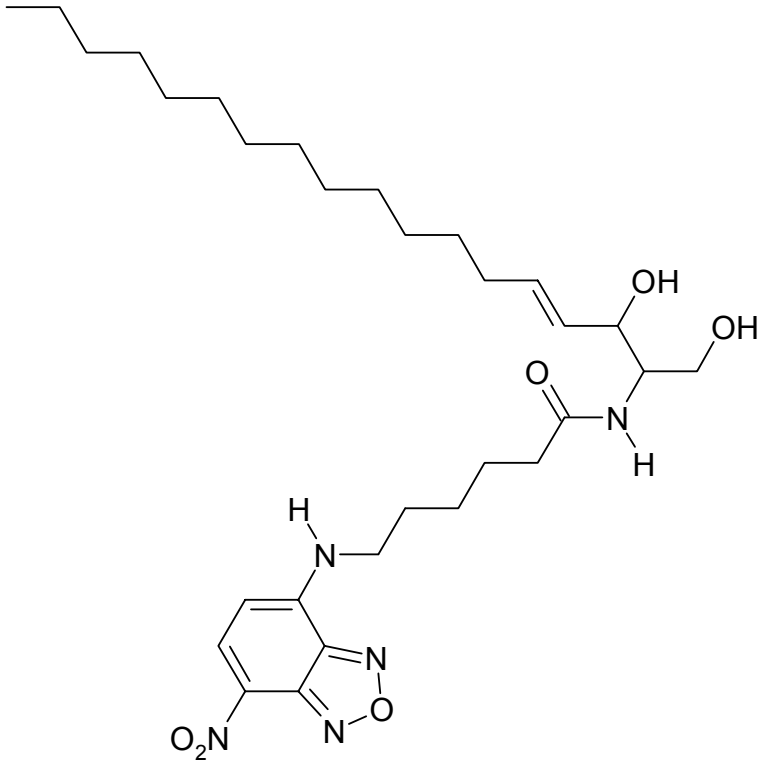


Figure 31: NBD-ceramide

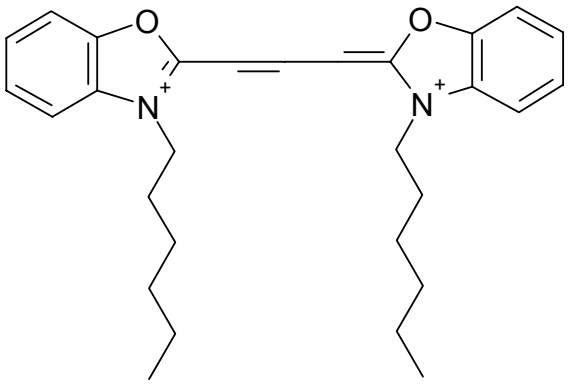


Figure 32: Carbocyanine dyes

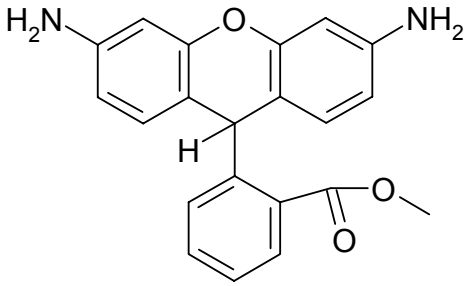


Figure 33: Dihydrorhodamine 123

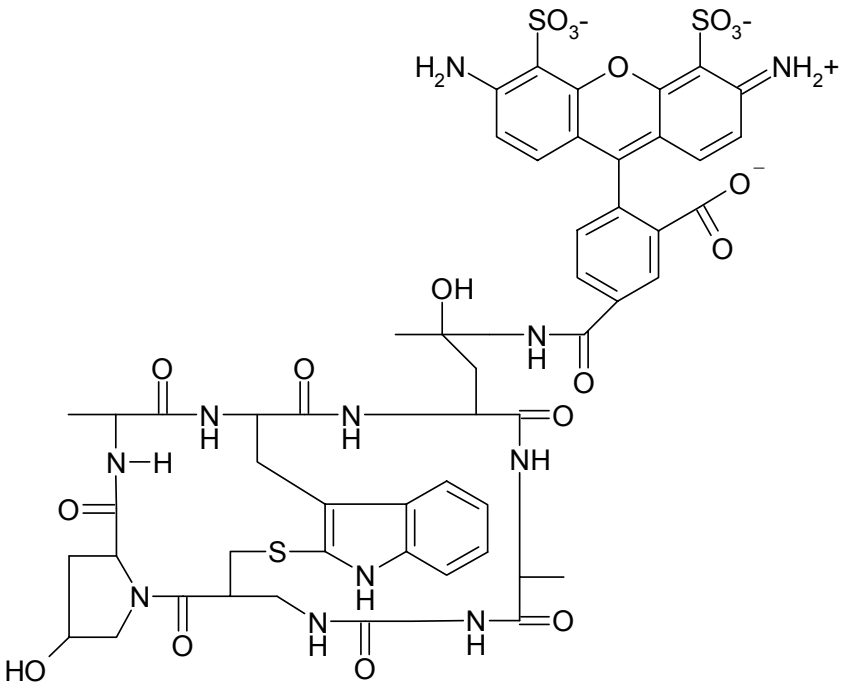


Figure 34: Alexa Fluor 488 phalloidin

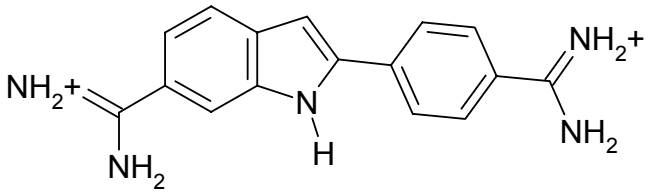


Figure 35: DAPI

Table 6[22]: Sub-cellular co-localization vector for targeted organelle

Targeted sub cellular structure	Dye Colors	Localization tag or gene	Potential application
Actin filaments	Green, yellow	Human β-actin	• study cytoskeletal dynamics • monitor co-localization associated proteins or organelles
Microtubles	Green, yellow	Human α-tubulin	• study cytoskeletal dynamics • monitor co-localization associated proteins or organelles
Mitochondria	Cyan, yellow, red	Targeting sequence from subunit VIII of cytochrome c oxidase	• study normal & disease state • track mitochondrial dynamics
Nucleus	Cyan, yellow	SV40 T-antigen NLS 3 tandem repeats	• study nuclear import • track cell lineage • monitor cell growth & division
Endoplasmic reticulum	Cyan, yellow	Targeting sequence of calreticulin: KDEL retrieval sequence	• visualize tubules & cisternae • track morphology & intracellular distribution
Golgi apparatus	Cyan , yellow	Targeting sequence from human beta 1: 4-galactosyltransferase	• study organelle dynamics • track morphology & intracellular distribution
Plasma membrane	Green, cyan, yellow	Palmitoylation domain of neuromodulin; farnesylation sequence from c-Ha-Ras (pEGFP-F)	• study membrane dynamic & protrusion • monitor membrane associated changes during apoptosis
Peroxisome	Green, cyan, yellow	Peroxisomal targeting signal 1 (PST1)	• monitor movement, segregation, biogenesis & degradation • study peroxisome purification

Method

The A7R5 cell line was obtained from the American Type Culture Collection and used at passage numbers 10-40. The cells were grown in Dulbecco's modified Eagles's medium (DMEM) containing 10% fetal bovine serum (FBS), 1 % penn strep. Tests for mycoplasmal contamination were not preformed. Cells were incubated in a humidified chamber at 37.7 ^{o}C with a mixture of 95% air and 5% carbon dioxide. Cells were grown on cover slips at 2 x10^5 cells per mL for 2 days. All dyes were dissolved into a DMEM to give a concentration of 3-5 mM. Cells to be imaged were allowed to grow in this dye-containing medium for 1 hour. MitoTracker RedXros® was added at 10 mM when co-localization studies were performed. Cells were imaged on a Bio-Rad MCR 1024 Confocal Microscope. The filters used in the Microscope were: 515 long pass filter, 522/35 band pass filter and 680/32 band pass filter.

Results

The PICs **1-16** (Figure 3) used in these experiments are violet excitable green fluorescent dyes. They have excitation maximum between 390-425 nm. In all cases these positively charged dyes had no difficulty in crossing the cellular membrane without the use of DMSO. Images obtained using confocal microscopy showed dyes **1-16** (Fig 37-48) had an affinity for imaging either ER/golgi and/or mitochondria. To more definitely ascertain their staining ability inside the cells. The cells were stained with the known mitochondria stain Mitrotracker red (Figure 36) in an effort to discriminate between ER/golgi and/or mitochondria as the target organelle. Comparisons were made

between the behavior of MitoTracker red[18] (Figure 36) and PICs **1-16**. Multiple dye experiments for four of these dyes were performed. Dyes **2a** (Figure 38), **1a** (Figure 40), **6a** (Figure 42), and **11b** (Figure 44) were proven to stain mitochondria through co-localization. All PICs **1-16** cross the plasma cellular membrane; they all also crossed the nuclear envelope. Dye **2a** (Figure 37), **1a** (Figure 43), and **11b** (Figure 45) revealed nuclear substructures.

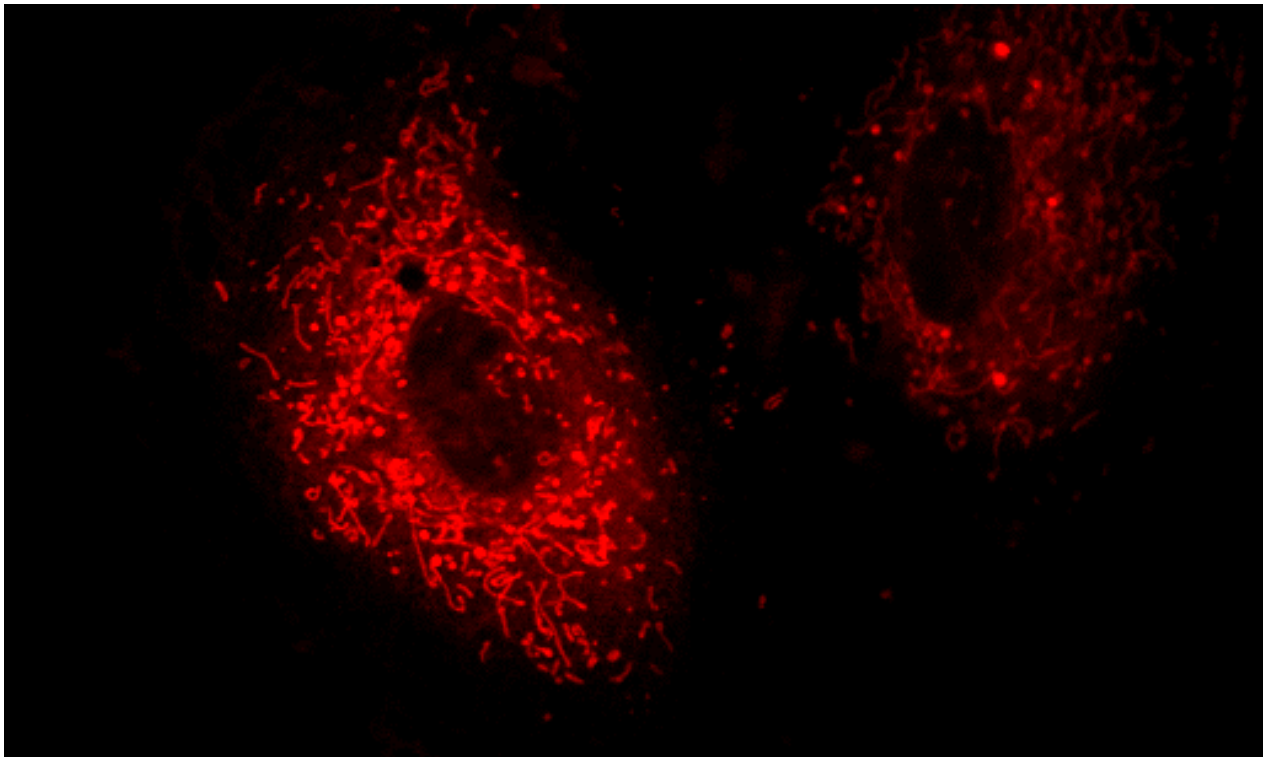


Figure 36: Mitrotracker red in A7R5

The picture in Figure 37 is of smooth muscle cells stained with dye **1a**. The small round objects appear to be the mitochondria. The objects illuminated are evenly spread out though the cell as expected for mitochondria

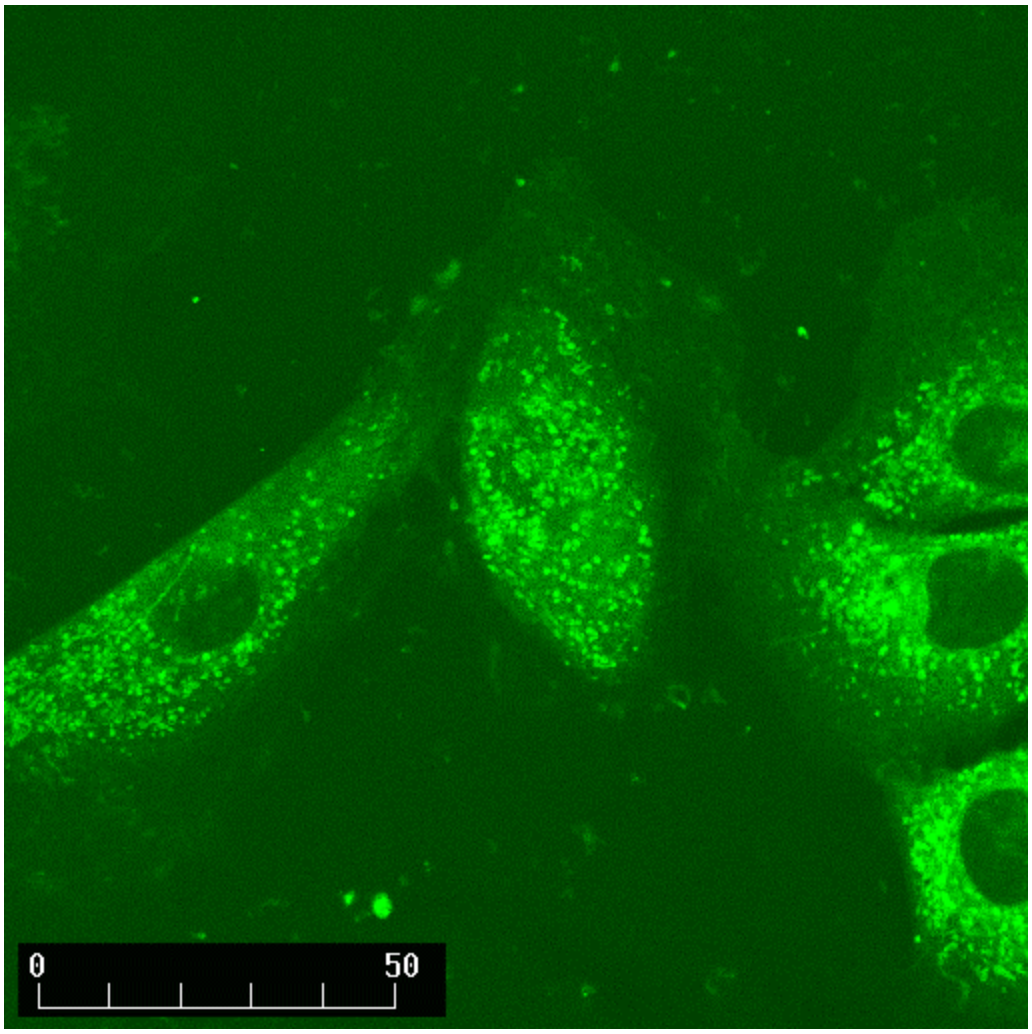


Figure 37: Dye 1a in smooth muscle cells of a rat's arota

Co-localization studies were conducted as follows: The commercially available dye, MitoTracker red, is known to stain live mitochondria. MitoTracker red has an emission maximum at 600 nm, which was collected in one channel. PICs have emission maximum of about 525 nm, which was collected in a separate channel. The data collected in each channel is in grayscale. MitoTracker red was given a superficial blue color, while the PICs were given a superficial red color. The MitoTracker and PICs channels were overlaid. If the small object in the Figure were present in both channels it would appear purple, and if it were only in one channel it would appear blue or red

depending on the channel. Most the small round objects appear purple, which confirms that **1a** does stain mitochondria (Figure 38). The objects in the nucleolus are stained from **1a**.

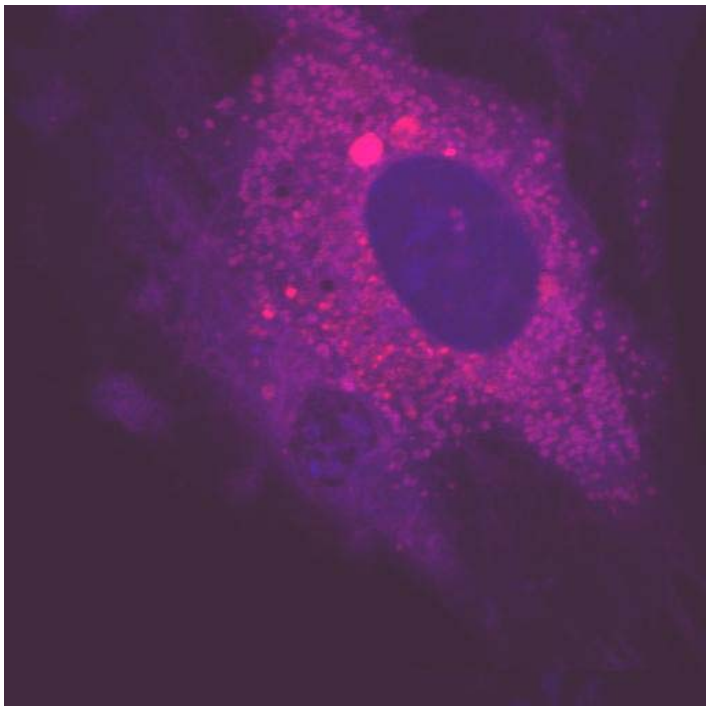


Figure 38: Dye 1a and MitoTracker red in smooth muscle cells of a rat's arota

The picture in Figure 39 is of smooth muscle cells stained with dye **11b**. It appears as though the relatively large football shape objects might be mitochondria along with the long strand structures.

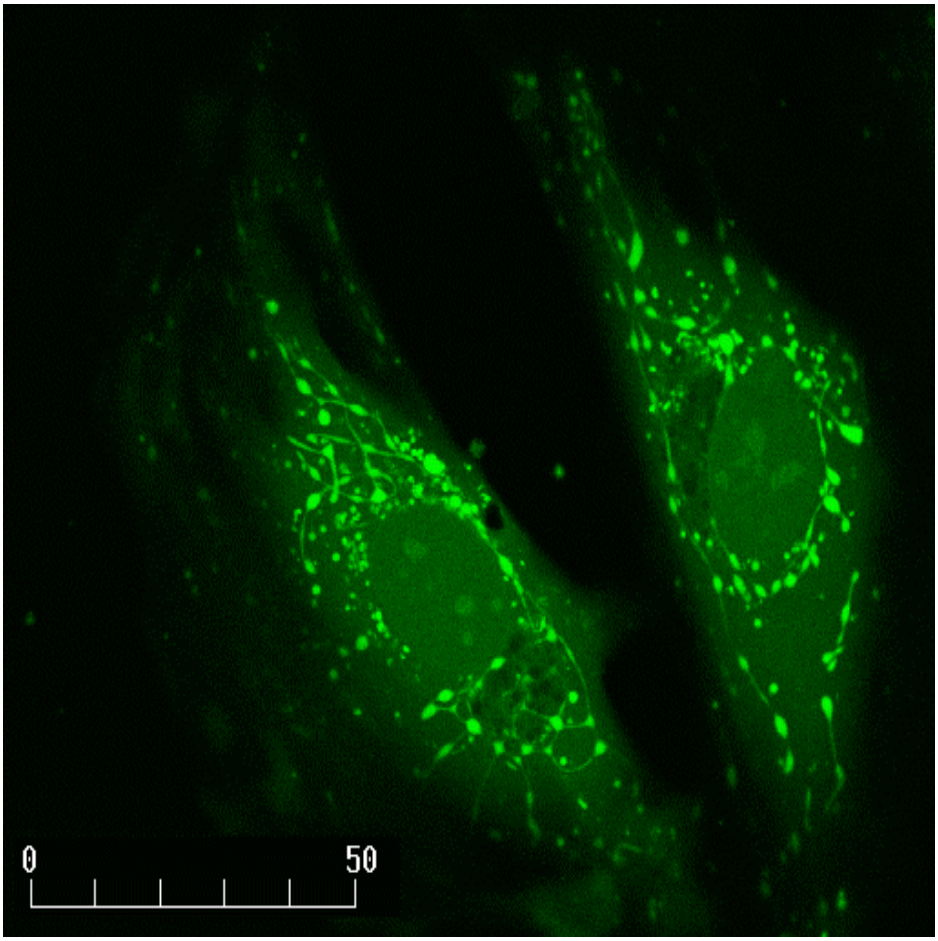


Figure 39: Dye 2a in smooth muscle cells of a rat's arota

The same method for co-localization was used as described above. Most of the small round objects appear purple, which confirms that dye **11b** does stain mitochondria (Figure 40).

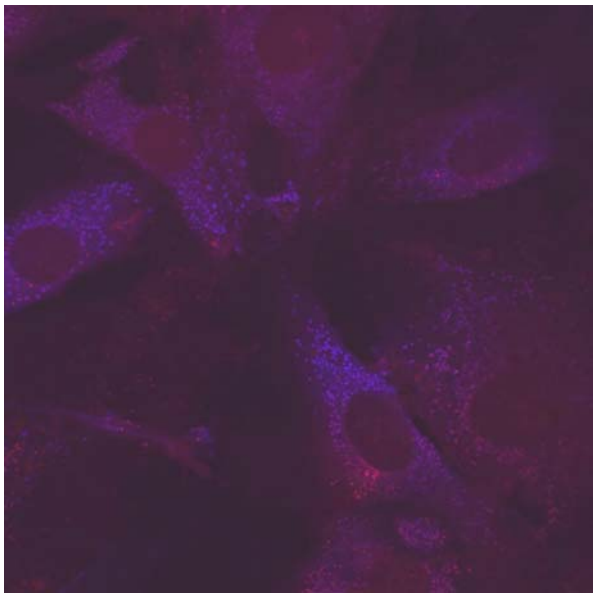


Figure 40: Dye 2a and MitoTracker red in smooth muscle cells of a rat's arota

The picture in Figure 41 is of smooth muscle cells stained with dye **1e**. Long strand-like objects are thought to be mitochondria.

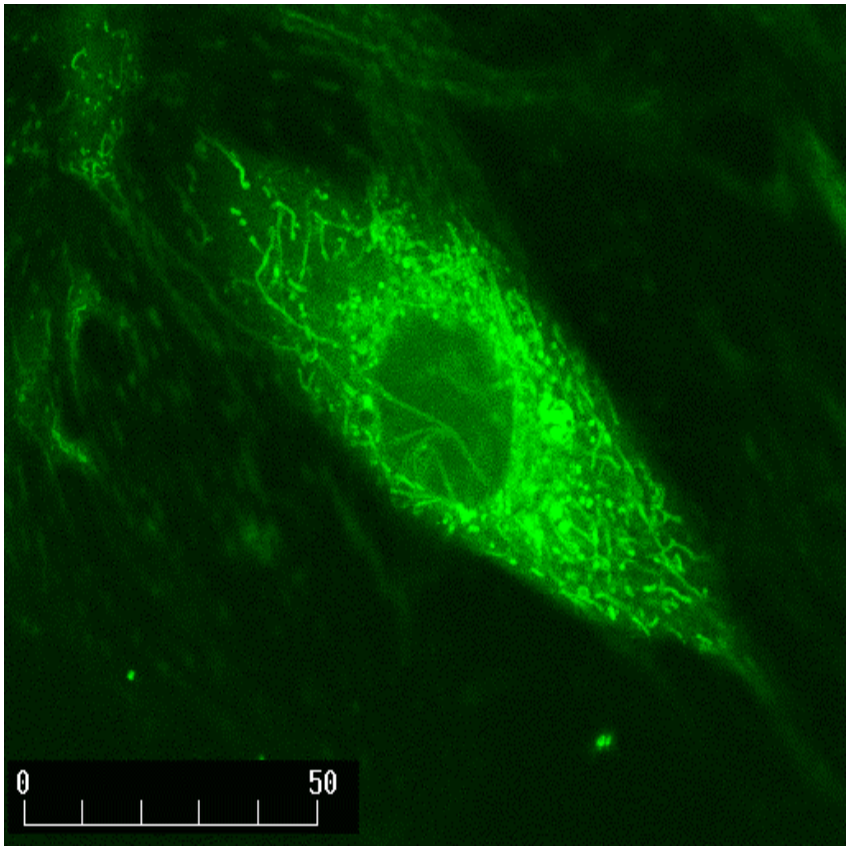


Figure 41: Dye 1e in smooth muscle cells of a rat's aorta

The same method for co-localization in smooth muscle cells of a rat's aorta was used as described above. The small round objects appear purple, which confirms that dye **6a** does stain mitochondria in Figure 42

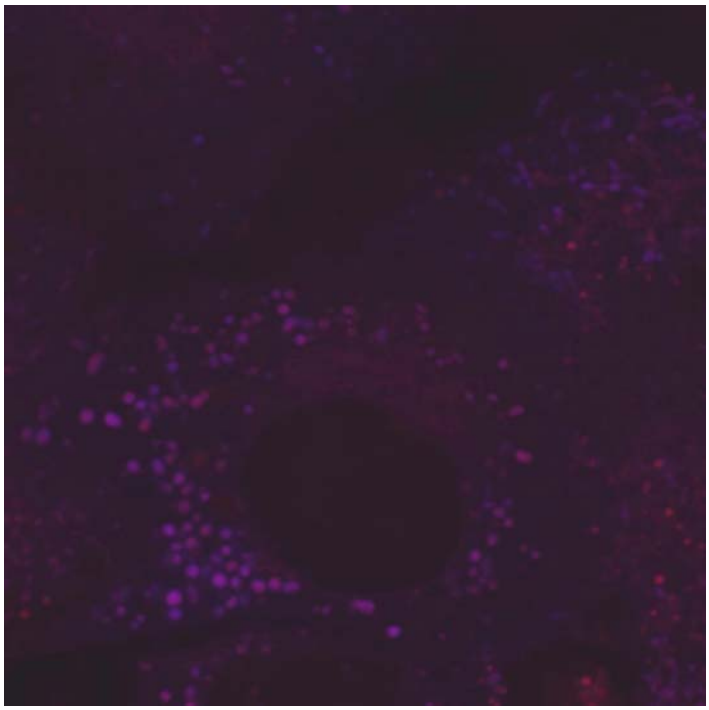


Figure 42: Dye 1e and MitoTracker red in smooth muscle cells of a rat's aorta

The picture in Figure 43 is of smooth muscle cells stained with dye **2a**. The long strand structures are thought to be mitochondria. The long strands are evenly spread out though the cell, as mitochondria should appear. The mitochondria brightness is much higher than the background.

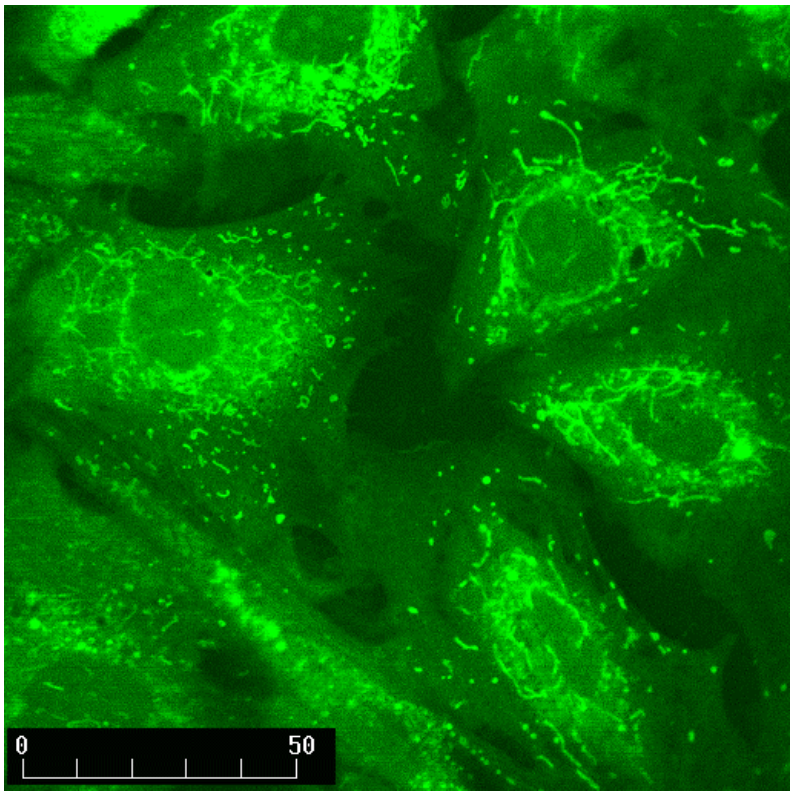


Figure 43: Dye 1b in smooth muscle cells of a rat's aorta

The same method for co-localization in smooth muscle cells of a rat's aorta was used as described above. Most the small round objects appear purple, which confirms that dye **2a** does stain mitochondria Figure 44

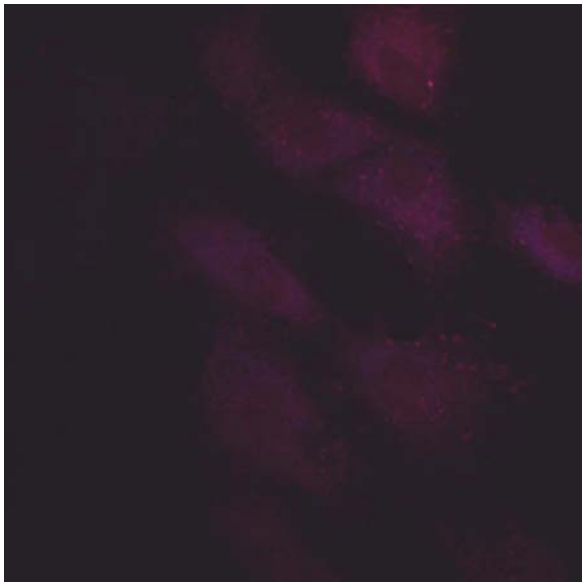


Figure 44: Dye 1b & MitoTracker red in smooth muscle cells of a rat's aorta

The picture in Figure 45 is of smooth muscle cells stained with dye **6a**. The long strand-like structures are thought to be mitochondria.

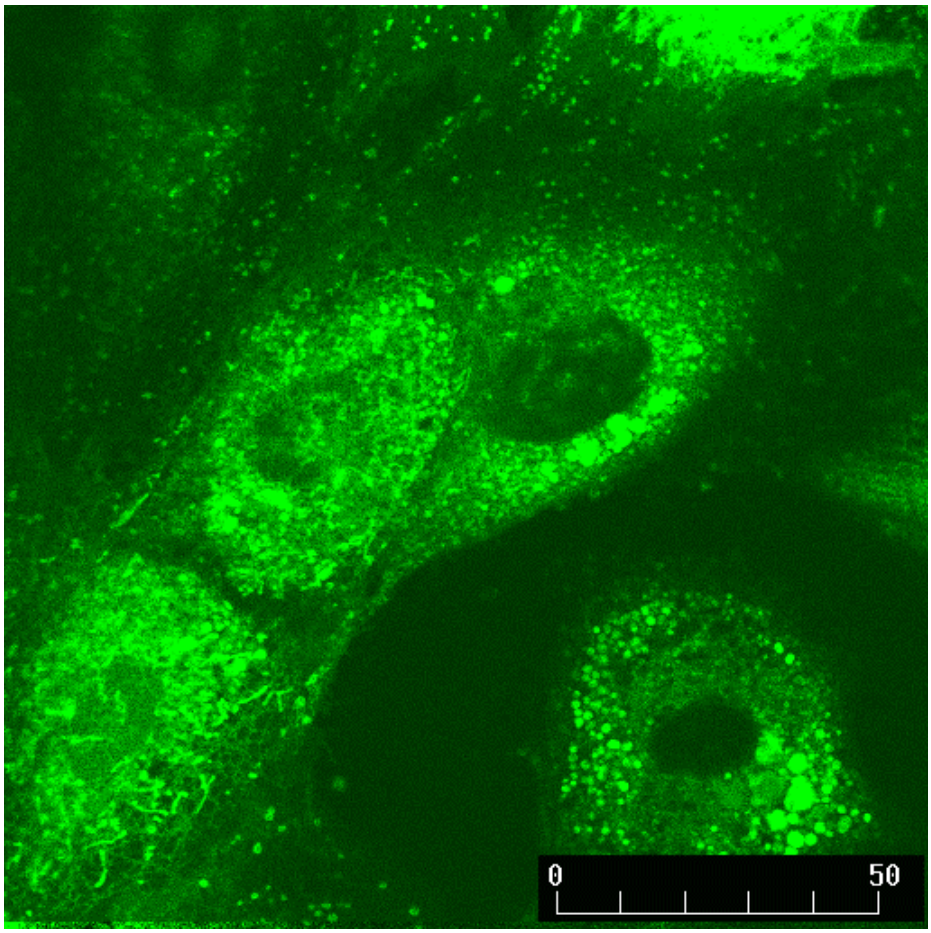


Figure 45: Dye 2e in smooth muscle cells of a rat's aorta

Dyes that are specific for nuclear envelopes are uncommon (lucifer yellow). The flexible methodology for synthesizing dyes allows for charge/structure relationships. Dyes **13b** (Figure 46) and **14b** (Figure 47) appeared to stain the nuclear envelope of the cells. Tri-cationic **14b** seems to have no difficulty crossing the plasma membrane and localizing in the nuclear envelope. Also interesting, dyes **13b** and **14b** are very similar in

structure, but are different in their charge (**13b** +1, **14b** +3). This would indicate that staining the nuclear envelopes in live cells is not charge dependent but structure dependent.

Dyes that are specific for nuclear envelopes in fixed cells are uncommon, but those that staining nuclear envelopes in live cells are rare. Once the dyes were proven to stain the nuclear envelope it was important to prove that the cells stained with **13b** were still viable. Two methods were used, which were staining with 4% trypan blue[23] and MitoTracker Red CM-H2Xros.[18] Trypan blue has no effect on living cells but renders dead cells or cells with a damaged cell membrane blue, as observed with an optical microscope. After 5 minutes, the cells were still colorless, demonstrating that they were neither damaged nor dead. MitoTracker Red CM-H2Xros is a non-luminescent stain, which will only fluoresce after being oxidized by the mitochondria, it then becomes a red fluorescent dye with an emission maximum at 600 nm. Emission at 600 nm was observed with MitoTracker Red CM-H2Xros stained cells, also indicating their vitality. To our knowledge dye **14b** is the first red fluorescent dye which stained the nuclear envelope in live cells. This ability to stain the nuclear envelope would be of great interest in co-staining live cells with other dyes. This dye may have uses in plant cells.

The picture in Figure 46 is of smooth muscle cells stained with dye **14b**. Dye **14b** specific stains nuclear envelope.

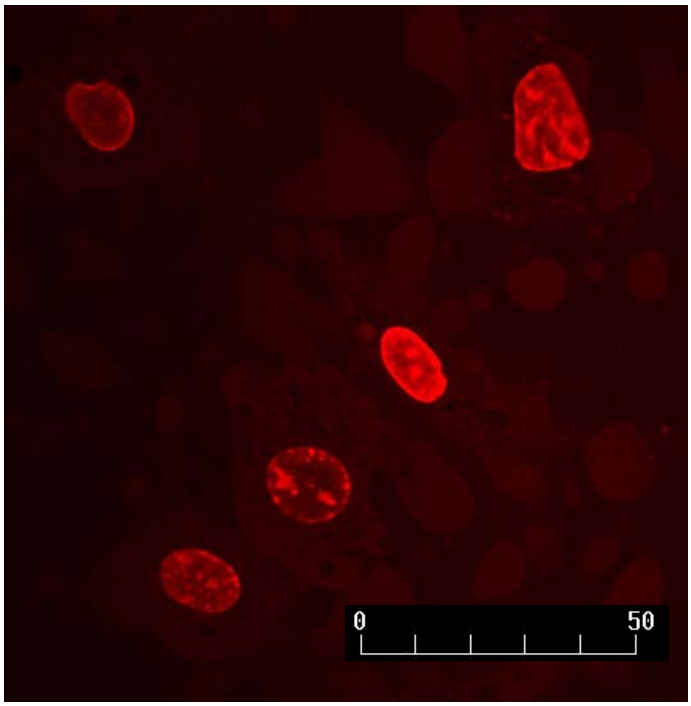


Figure 46: Dye 2d in smooth muscle cells of a rat's aorta

The picture in Figure 47 is of smooth muscle cells stained with dye **13b**. Dye **14b** seems to locate around the nuclear envelope, although there are is dye in the cytoplasm.

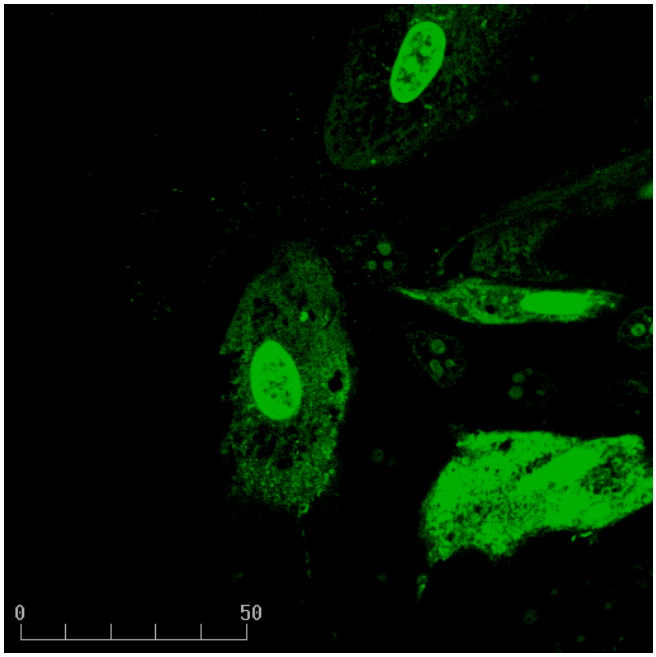


Figure 47: Dye 2c in smooth muscle cells of a rat's aorta

Conclusion

The behavior of green fluorescent PICs in live smooth muscle cells was probed using confocal microscopy. Dyes **2a**, **1a**, **6a**, and **11b** were proven to stain mitochondria through co-localization. All PICs 1 and 2 cross the plasma cellular membrane, they all also crossed the nuclear envelope. Dye **2a**, **1a**, and **11b** revealed nuclear substructures. Dyes **13b** and **14b** are very similar in structure, but are different in their charge (**13b** +1, **14b** +3). This would indicate that staining the nuclear envelopes in live cells is structure dependent not charge dependent. To our knowledge dye **14b** is the first red fluorescent dye that has been shown to stain the nuclear envelope in live cells.

Chapter 4: Summary and Conclusion

Pyridoimidazolium cations (PICs) are a new class of fluorescent dyes, which are prepared by an exceedingly flexible methodology. Using computational chemical methods the excitation and emission maxima of these dyes were simulated. This was best accomplished through geometry optimization (MM+) followed by calculating the electronic spectra with ZINDO/S. This protocol has been found to be effective and has the potential to greatly streamline the design of new longer wavelength dyes. New longer wavelength dyes have now been identified as synthetic targets, **16b**.

The presences of amino groups on a known fluorescent ring system are well established to markedly affect the optical properties of the dye. Methodology had been developed for the amination of different position on the 2-(2-pyridyl)-carboxyquinoline ring system(4-Carboxymethyl-6-amino-2-(2-pyridyl)quinoline,**8a**, and 4-Carboxymethyl-8-amino-2-(2-pyridyl)quinoline), leading to the testing of three new amino substituted fluorescent dyes. Another methodology was effect in synthesis of 4-amino-2-(2-pyridyl)quinoline, **6a.** This is also the precursor to 4-isothiocyanato-amino-2-(2-pyridyl)quinoline, which can be used as a cellular tag for proteins.

The behavior of green fluorescent PICs in live smooth muscle cells, was probed using confocal microscopy. Dyes **2a**, **1a**, **6a**, and **11b** were proven to stain mitochondria through co-localization. All PICs 1 and 2 cross the plasma cellular membrane, they all also crossed the nuclear envelope. Dye **2a**, **1a**, and **11b** revealed nuclear substructures. Dyes **13b** and **14b** are very similar in structure, but are different in their charge (**13b** +1,

14b +3). This would indicate that staining the nuclear envelopes in live cells is structure dependent not charge dependent. To our knowledge dye **14b** is the first red fluorescent dye that has been shown to stain the nuclear envelope in live cells.

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Appendix I

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HyperChem log start -- Mon Jul 01 20:43:36 2002.
Geometry optimization, MolecularMechanics, molecule = C:\My
Documents\Hyper chem files\cincacid.hin.
mmplus
PolakRibiere optimizer
Default parameters being used for torsions...
Default parameters being used for bends...
Energy=25.515825 Gradient=0.092821 Converged=YES (0 cycles 1 points).
Bond=1.84261   Angle=19.4408   Dihedral=-6.27828 Vdw=17.6903   Stretch-
bend=0.286719   Electrostatic=-7.46631.
Single Point, SemiEmpirical, molecule = C:\My Documents\Hyper chem
files\cincacid.hin.
ZINDOS
Convergence limit = 0.0100000  Iteration limit = 50
Overlap weighting factors: P(Sigma-Sigma) = 1.2670 and P(Pi-Pi) =
0.5850
Accelerate convergence = YES
RHF Calculation:

Singlet state calculation
Configuration interaction will be used
Number of electrons = 114
Number of Double Occupied Levels = 57
Charge on the System = 1
Total Orbitals = 108
Number of Occupied Orbitals Used in CI = 5
Number of Unoccupied Orbitals Used in CI = 5

Starting ZINDO/S calculation with 108 orbitals

Iteration = 1 Difference = 67452.50648
Iteration = 2 Difference = 375.77163
Iteration = 3 Difference = 34.03356
Iteration = 4 Difference = 19.82689
Iteration = 5 Difference = 24.72793
Iteration = 6 Difference = 0.28406
Iteration = 7 Difference = 0.10147
Iteration = 8 Difference = 0.01821
Iteration = 9 Difference = 0.00683
Starting a singly excited CI calculation with 51 configurations.
Computing the integrals for CI matrix: done 0%.
Computing the integrals for CI matrix: done 10%.
Computing the integrals for CI matrix: done 20%.
Computing the integrals for CI matrix: done 30%.
Computing the integrals for CI matrix: done 40%.
Computing the integrals for CI matrix: done 50%.
Computing the integrals for CI matrix: done 60%.
Computing the integrals for CI matrix: done 70%.
Computing the integrals for CI matrix: done 80%.
Computing the integrals for CI matrix: done 90%.
Computing the integrals for CI matrix: done 100%.
Computing the CI matrix...
Diagonalizing the CI matrix...
```

```
Computing the properties of the CI states...
                  *********************************
                  ********** UV Spectrum **********
                  *********************************

 ---- Absolute Energy in eV.
 ---- Dipole Moments in Debye.


   0 (Reference)  Absolute Energy     -4439.31592

            1     Spin S                  0.00
                  State Dipole            7.8538

                  State Dipole Components         7.2633   -2.9508   -
0.4692


   1 (Transition) Excitation Energy         921.8 nm
                                          10848.4 1/cm

        1 ->   2  Spin S                 1.00
                  State Dipole            9.3690
                  Oscillator Strength     0.0000

                  State Dipole Components   8.9026   -2.8550   -0.6081
                  Transition Dipole Components -0.0000 0.0000    0.0000

        Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
       -------------------------------------------------
                     57  -->    58            0.658679

       Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
       -------------------------------------------------
                     57  -->    58            0.658679


   2 (Transition) Excitation Energy         670.1 nm
                                          14922.6 1/cm

        1 ->   3  Spin S                 1.00
                  State Dipole            5.2560
                  Oscillator Strength     0.0000

                  State Dipole Components   3.6432   -3.7866   -0.1222
                  Transition Dipole Components 0.0000 -0.0000    0.0000

        Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
       -------------------------------------------------
                     57  -->    59           -0.574307

       Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
       -------------------------------------------------
                     57  -->    59           -0.574307
```

```
3 (Transition) Excitation Energy         519.1 nm
                                       19263.5 1/cm

     1 ->   4  Spin S                 1.00
               State Dipole            7.6766
               Oscillator Strength     0.0000

               State Dipole Components    4.6532   -6.1048   -0.0902
               Transition Dipole Components 0.0000  0.0000   -0.0000

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    -------------------------------------------------
                   57  -->    60           -0.550858

    Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
    -------------------------------------------------
                   57  -->    60           -0.550858


4 (Transition) Excitation Energy         433.7 nm
                                       23058.5 1/cm

     1 ->   5  Spin S                 0.00
               State Dipole            9.0062
               Oscillator Strength     0.7163

               State Dipole Components    7.3561   -5.1835   -0.3612
               Transition Dipole Components 8.0882 -0.4876   -0.6383

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    -------------------------------------------------
                   57  -->    58            0.691536

    Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
    -------------------------------------------------
                   57  -->    58           -0.691536


5 (Transition) Excitation Energy         409.4 nm
                                       24428.8 1/cm

     1 ->   6  Spin S                 1.00
               State Dipole            7.0557
               Oscillator Strength     0.0000

               State Dipole Components    5.8033   -4.0018   -0.2994
               Transition Dipole Components  0.0000 0.0000   -0.0000

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    -------------------------------------------------
                   56  -->    58            0.408779
```

```
    Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  56  -->    58            0.408779


6 (Transition) Excitation Energy         394.4 nm
                                        25354.1 1/cm
     1 ->   7  Spin S                  0.00
               State Dipole             8.0368
               Oscillator Strength      0.1752

               State Dipole Components   7.0122   -3.9071   -0.3929
               Transition Dipole Components -2.0240 -3.2373  0.3433

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  57  -->    59            0.648244

    Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  57  -->    59           -0.648244


7 (Transition) Excitation Energy         372.8 nm
                                        26825.0 1/cm
     1 ->   8  Spin S                  1.00
               State Dipole             8.5066
               Oscillator Strength      0.0000

               State Dipole Components    8.2335   -2.0550   -0.5909
               Transition Dipole Components  0.0000  0.0000   -.0000

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  57  -->    61           -0.548251
                  55  -->    59           -0.337136

    Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  57  -->    61           -0.548251
                  55  -->    59           -0.337136


8 (Transition) Excitation Energy         335.7 nm
                                        29788.7 1/cm
     1 ->   9  Spin S                  1.00
               State Dipole            10.4357
               Oscillator Strength      0.0000

               State Dipole Components    9.3946   -4.5070   -0.5766
               Transition Dipole Components -0.0000 -0.0000   0.0000

     Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
    ------------------------------------------------
                  56  -->    58           -0.387314
```

```
                    55  -->    58            0.441698

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    56  -->    58           -0.387314
                    55  -->    58            0.441698

 9 (Transition) Excitation Energy         326.4 nm
                                         30639.1 1/cm

      1 ->  10  Spin S                 0.00
                State Dipole            5.5655
                Oscillator Strength     0.0061

                State Dipole Components    3.0015   -4.6867   -0.0302
                Transition Dipole Components -0.3873  0.5223   0.0038

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    57  -->    60           -0.667004

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    57  -->    60            0.667004


10 (Transition) Excitation Energy         326.2 nm
                                         30658.3 1/cm

      1 ->  11  Spin S                 1.00
                State Dipole            5.9428
                Oscillator Strength     0.0000

                State Dipole Components   -2.8240    5.2289   -0.0360
                Transition Dipole Components  0.0000  0.0000  0.0000

    Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    53  -->    58           -0.604259

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    53  -->    58           -0.604259


11 (Transition) Excitation Energy         323.0 nm
                                         30957.7 1/cm

      1 ->  12  Spin S                 1.00
                State Dipole            8.8837
                Oscillator Strength     0.0000

                State Dipole Components    5.1165   -7.2618   -0.0854
                Transition Dipole Components  0.0000 -0.0000   -.0000
```

```
       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    59            0.494328
                    56  -->    61            0.365019

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    59            0.494328
                    56  -->    61            0.365019
 12 (Transition) Excitation Energy         313.3 nm
                                         31916.3 1/cm

       1 ->  13  Spin S                 0.00
                 State Dipole            6.0425
                 Oscillator Strength     0.0002
                 State Dipole Components   -2.9240    5.2878   -0.0308
                 Transition Dipole Components -0.0132 0.0106  -0.1142

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    58           -0.609937

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    58            0.609937

 13 (Transition) Excitation Energy         300.6 nm
                                         33261.7 1/cm

       1 ->  14  Spin S                 0.00
                 State Dipole            6.9091
                 Oscillator Strength     0.4343
                 State Dipole Components    4.6467   -5.1107   -0.1564
                 Transition Dipole Components -3.3867 -4.0068  0.4930

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    57  -->    61           -0.411881
                    56  -->    58            0.422232

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    57  -->    61            0.411881
                    56  -->    58           -0.422232

 14 (Transition) Excitation Energy         299.2 nm
                                         33425.1 1/cm

       1 ->  15  Spin S                 1.00
                 State Dipole           11.3815
                 Oscillator Strength     0.0000
                 State Dipole Components    9.1455   -6.7628   -0.4028
                 Transition Dipole Components 0.0000  0.0000   -0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
```

```
      ------------------------------------------------
                57  -->    62           -0.405065
                55  -->    58           -0.351218

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                57  -->    62           -0.405065
                55  -->    58           -0.351218


15 (Transition) Excitation Energy         298.3 nm
                                          33527.3 1/cm

       1 ->  16  Spin S                 1.00
                 State Dipole           16.3314
                 Oscillator Strength     0.0000
                 State Dipole Components   11.9267  -11.1524   -0.3036
                 Transition Dipole Components 0.0000  0.0000  -0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                54  -->    58            0.461115
                54  -->    59            0.403452

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                54  -->    58            0.461115
                54  -->    59            0.403452


  16 (Transition) Excitation Energy         297.3 nm
                                           33635.6 1/cm

       1 ->  17  Spin S                 0.00
                 State Dipole           17.1313
                 Oscillator Strength     0.0047
                 State Dipole Components   12.1779  -12.0471   -0.2198
                 Transition Dipole Components -0.3060 -0.4515   0.0197

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                54  -->    58            0.490264
                54  -->    59            0.426896

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                54  -->    58           -0.490264
                54  -->    59           -0.426896

  17 (Transition) Excitation Energy         293.2 nm
                                           34101.1 1/cm

       1 ->  18  Spin S                 0.00
                 State Dipole            9.3148
```

```
                Oscillator Strength     0.0085
                State Dipole Components    3.7398   -8.5307    0.0758
                Transition Dipole Components 0.0995 -0.7225    0.0216

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   56  -->    58            0.393011
                   55  -->    58            0.428684

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   56  -->    58           -0.393011
                   55  -->    58           -0.428684
 18 (Transition) Excitation Energy         277.5 nm
                                         36033.5 1/cm

      1 ->  19  Spin S                 1.00
                State Dipole            7.9001
                Oscillator Strength     0.0000
                State Dipole Components    7.1327   -3.3680   -0.4393
                Transition Dipole Components -0.0000 -0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   57  -->    61           -0.350414
                   55  -->    59            0.481186

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   57  -->    61           -0.350414
                   55  -->    59            0.481186


 19 (Transition) Excitation Energy         273.6 nm
                                         36551.6 1/cm

      1 ->  20  Spin S                 0.00
                State Dipole           11.9409
                Oscillator Strength     0.3367
                State Dipole Components   11.6706   -2.3730   -0.8674
                Transition Dipole Components  0.5430  4.3842  -0.2675

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   57  -->    61           -0.365808
                   55  -->    58            0.486533

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   57  -->    61            0.365808
                   55  -->    58           -0.486533

 20 (Transition) Excitation Energy         265.6 nm
                                         37655.9 1/cm
```

```
       1 ->  21  Spin S                 -0.00
                 State Dipole           12.6826
                 Oscillator Strength     0.1952
                 State Dipole Components   12.4304   -2.3425   -0.9204
                 Transition Dipole Components 3.3018 -0.2322   -0.2606

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    57  -->    62            0.488568
                    56  -->    59           -0.342710

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    57  -->    62           -0.488568
                    56  -->    59            0.342710
 21 (Transition) Excitation Energy         258.4 nm
                                         38706.6 1/cm

       1 ->  22  Spin S                 1.00
                 State Dipole           10.3085
                 Oscillator Strength     0.0000
                 State Dipole Components   8.6672   -5.5621   -0.4571
                 Transition Dipole Components -0.0000 -0.0000   0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    62            0.497894

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    62            0.497894


 22 (Transition) Excitation Energy         250.4 nm
                                         39931.0 1/cm

       1 ->  23  Spin S                 0.00
                 State Dipole            6.0891
                 Oscillator Strength     0.6878
                 State Dipole Components   2.3728   -5.6074    0.0607
                 Transition Dipole Components -5.9351 -1.0639   0.5247

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    59            0.518637
                    55  -->    59           -0.342089

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    59           -0.518637
                    55  -->    59            0.342089


 23 (Transition) Excitation Energy         244.8 nm
                                         40847.3 1/cm
```

```
      1 ->  24  Spin S                 1.00
                State Dipole           12.8386
                Oscillator Strength     0.0000
                State Dipole Components  2.0462  -12.6630    0.5415
                Transition Dipole Components -0.0000 -0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   55  -->    60           -0.360460
                   54  -->    58           -0.333624

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   55  -->    60           -0.360460
                   54  -->    58           -0.333624

24 (Transition) Excitation Energy         244.2 nm
                                         40946.6 1/cm

      1 ->  25  Spin S                 1.00
                State Dipole           13.9802
                Oscillator Strength     0.0000
                State Dipole Components    3.0993  -13.6234    0.4935
                Transition Dipole Components  0.0000  0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   55  -->    60            0.337826
                   54  -->    58           -0.368394

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   55  -->    60            0.337826
                   54  -->    58           -0.368394


25 (Transition) Excitation Energy         244.1 nm
                                         40964.7 1/cm

      1 ->  26  Spin S                 0.00
                State Dipole           19.7618
                Oscillator Strength     0.0020
                State Dipole Components   8.7107  -17.7351    0.3464
                Transition Dipole Components -0.0333 -0.3092 -0.0790

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   54  -->    58           -0.492916
                   54  -->    59            0.391990

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   54  -->    58            0.492916
                   54  -->    59           -0.391990
```

```
26 (Transition) Excitation Energy         242.4 nm
                                        41259.3 1/cm

      1 ->  27  Spin S                 0.00
                State Dipole            7.6399
                Oscillator Strength     0.3868
                State Dipole Components    6.6459   -3.7486   -0.3843
                Transition Dipole Components 1.4239  4.2191   -0.3270

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   57  -->    62           -0.386954
                   55  -->    59           -0.465023

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   57  -->    62            0.386954
                   55  -->    59            0.465023
27 (Transition) Excitation Energy         236.3 nm
                                        42319.7 1/cm

      1 ->  28  Spin S                 1.00
                State Dipole            6.2328
                Oscillator Strength     0.0000
                State Dipole Components   4.1044   -4.6888   -0.1284
                Transition Dipole Components 0.0000  0.0000   -0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   56  -->    61            0.434468
                   55  -->    61           -0.462556

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   56  -->    61            0.434468
                   55  -->    61           -0.462556


28 (Transition) Excitation Energy         219.3 nm
                                        45605.5 1/cm

      1 ->  29  Spin S                -0.00
                State Dipole            9.5707
                Oscillator Strength     0.0155
                State Dipole Components  -1.9660   -9.3474    0.6004
                Transition Dipole Components 0.2013 0.8225   -0.0742

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   56  -->    60            0.460575
                   55  -->    60           -0.455662

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
```

```
                    56  -->    60           -0.460575
                    55  -->    60            0.455662


29 (Transition) Excitation Energy         215.7 nm
                                         46370.6 1/cm

      1 ->  30  Spin S                  1.00
                State Dipole           14.4936
                Oscillator Strength     0.0000
                State Dipole Components  -8.9709  -11.3125    1.2717
                Transition Dipole Components -0.0000 -0.0000  0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    55  -->    61           -0.411812

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    55  -->    61           -0.411812

30 (Transition) Excitation Energy         214.0 nm
                                         46723.9 1/cm

      1 ->  31  Spin S                  0.00
                State Dipole            8.4550
                Oscillator Strength     0.0926
                State Dipole Components   -1.4807   -8.3087    0.5093
                Transition Dipole Components -1.9820 -0.4987   0.1963

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    56  -->    62            0.377585
                    55  -->    61            0.326276

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    56  -->    62           -0.377585
                    55  -->    61           -0.326276

31 (Transition) Excitation Energy         207.1 nm
                                         48280.5 1/cm

      1 ->  32  Spin S                  1.00
                State Dipole           21.2254
                Oscillator Strength     0.0000
                State Dipole Components  10.5179  -18.4345    0.2475
                Transition Dipole Components 0.0000 -0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                    54  -->    59            0.388435
                    54  -->    60           -0.580129

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
```

```
     ------------------------------------------------
                  54  -->    59            0.388435
                  54  -->    60           -0.580129


32 (Transition) Excitation Energy         207.0 nm
                                         48301.1 1/cm

      1 ->  33  Spin S                 0.00
                State Dipole           21.3257
                Oscillator Strength     0.0001
                State Dipole Components   10.6679  -18.4643    0.2324
                Transition Dipole Components -0.0079 -0.0753   0.0270

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                  54  -->    59            0.388869
                  54  -->    60           -0.583079

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                  54  -->    59           -0.388869
                  54  -->    60            0.583079
33 (Transition) Excitation Energy         205.5 nm
                                         48650.1 1/cm

      1 ->  34  Spin S                 1.00
                State Dipole           19.3534
                Oscillator Strength     0.0000
                State Dipole Components -11.3972  -15.5518    1.6724
                Transition Dipole Components -0.0000 -0.0000  0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                  56  -->    60            0.434429

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                  56  -->    60            0.434429


34 (Transition) Excitation Energy         204.7 nm
                                         48862.5 1/cm

      1 ->  35  Spin S                 0.00
                State Dipole           16.0128
                Oscillator Strength     0.0199
                State Dipole Components   -8.1162  -13.7405    1.3167
                Transition Dipole Components 0.4336  0.8199   -0.0645

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                  56  -->    60            0.368854
                  55  -->    60            0.457856
```

```
      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    60           -0.368854
                    55  -->    60           -0.457856


  35 (Transition) Excitation Energy         204.2 nm
                                           48961.4 1/cm

       1 ->  36  Spin S                 1.00
                 State Dipole           13.5926
                 Oscillator Strength     0.0000
                 State Dipole Components   -8.1714   10.8613    0.1357
                 Transition Dipole Components -0.0000 -0.0000   0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    59           -0.633303

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    59           -0.633303


36 (Transition) Excitation Energy         204.1 nm
                                           48994.1 1/cm

       1 ->  37  Spin S                 0.00
                 State Dipole           13.5959
                 Oscillator Strength     0.0005
                 State Dipole Components   -8.2300   10.8211    0.1413
                 Transition Dipole Components -0.0021 -0.0107   0.1474

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    59            0.633182

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    59           -0.633182


  37 (Transition) Excitation Energy         203.7 nm
                                           49099.5 1/cm

       1 ->  38  Spin S                 1.00
                 State Dipole           23.0075
                 Oscillator Strength     0.0000
                 State Dipole Components   22.5019    4.3204   -2.0843
                 Transition Dipole Components -0.0000 -0.0000   0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    55  -->    62           -0.586248
```

```
      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    55  -->    62           -0.586248


 38 (Transition) Excitation Energy         198.5 nm
                                          50381.1 1/cm

       1 ->  39  Spin S                 0.00
                 State Dipole            6.9082
                 Oscillator Strength     0.0530
                 State Dipole Components    2.3015   -6.5126    0.1136
                 Transition Dipole Components  -0.6520  1.3455 -0.0007

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    61           -0.562009

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    61            0.562009




39 (Transition) Excitation Energy         188.6 nm
                                          53030.3 1/cm

       1 ->  40  Spin S                 0.00
                 State Dipole            8.2867
                 Oscillator Strength     0.8893
                 State Dipole Components    7.6692   -3.0995   -0.4959
                 Transition Dipole Components 3.1292  -5.0864  0.0025

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    62           -0.398695
                    55  -->    61            0.517145

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    56  -->    62            0.398695
                    55  -->    61           -0.517145


 40 (Transition) Excitation Energy         187.6 nm
                                          53305.9 1/cm

       1 ->  41  Spin S                 1.00
                 State Dipole           20.8411
                 Oscillator Strength     0.0000
                 State Dipole Components   17.8939  -10.6575   -0.7593
                 Transition Dipole Components -0.0000  0.0000  0.0000
```

```
       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     54  -->    61           -0.659549

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     54  -->    61           -0.659549


  41 (Transition) Excitation Energy         187.4 nm
                                           53348.7 1/cm

       1 ->  42  Spin S                 -0.00
                 State Dipole           20.6984
                 Oscillator Strength     0.0095

                 State Dipole Components   17.7682  -10.5898   -0.7574
                 Transition Dipole Components -0.3396  0.5116  0.0305

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     54  -->    61            0.657516

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     54  -->    61           -0.657516


  42 (Transition) Excitation Energy         183.5 nm
                                           54494.3 1/cm

       1 ->  43  Spin S                 -0.00
                 State Dipole           15.8948
                 Oscillator Strength     0.3150
                 State Dipole Components   15.7803    1.3068   -1.3849
                 Transition Dipole Components  2.5612  2.3736  -0.3120

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     55  -->    62           -0.604705

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                     55  -->    62            0.604705


  43 (Transition) Excitation Energy         182.3 nm
                                           54862.6 1/cm

       1 ->  44  Spin S                 1.00
                 State Dipole           14.0025
                 Oscillator Strength     0.0000
                 State Dipole Components   -8.7520   10.9288    0.1856
Transition Dipole Components    0.0000    0.0000   -0.0000
```

```
      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60            0.365687
                   53  -->    62            0.477004

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60            0.365687
                   53  -->    62            0.477004


44 (Transition) Excitation Energy         181.6 nm
                                        55069.3 1/cm

      1 ->  45  Spin S                 -0.00
                State Dipole           14.0987
                Oscillator Strength     0.0002
                State Dipole Components   -9.4458   10.4633    0.2638
                Transition Dipole Components -0.0602  -0.0619  0.0354

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60            0.391470
                   53  -->    62            0.466529

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60           -0.391470
                   53  -->    62           -0.466529


45 (Transition) Excitation Energy         175.2 nm
                                        57087.2 1/cm

      1 ->  46  Spin S                 1.00
                State Dipole           15.4054
                Oscillator Strength     0.0000
                State Dipole Components  -10.1556   11.5810    0.2650
                Transition Dipole Components -0.0000 -0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60            0.538937

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     ------------------------------------------------
                   53  -->    60            0.538937


46 (Transition) Excitation Energy         175.0 nm
                                        57129.5 1/cm

      1 ->  47  Spin S                 0.00
                State Dipole           15.2057
```

```
                Oscillator Strength     0.0000
                State Dipole Components   -9.3939   11.9555    0.1841
                Transition Dipole Components  0.0056  0.0073  -0.0246

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   53  -->    60            0.522332
                   53  -->    62           -0.331347

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   53  -->    60           -0.522332
                   53  -->    62            0.331347


47 (Transition) Excitation Energy         173.0 nm
                                        57794.0 1/cm

      1 ->  48  Spin S                 1.00
                State Dipole           27.3586
                Oscillator Strength     0.0000
                State Dipole Components   25.8827   -8.7344   -1.5127
                Transition Dipole Components  0.0000  0.0000   0.0000

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   54  -->    62           -0.661222

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   54  -->    62           -0.661222


48 (Transition) Excitation Energy         172.9 nm
                                        57852.5 1/cm

      1 ->  49  Spin S                 -0.00
                State Dipole           27.3786
                Oscillator Strength     0.0002
                State Dipole Components   25.9117   -8.7103   -1.5169
                Transition Dipole Components  0.0637 -0.0096   0.0495

      Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   54  -->    62            0.662286

     Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
     -------------------------------------------------
                   54  -->    62           -0.662286


49 (Transition) Excitation Energy         165.7 nm
                                        60344.6 1/cm

      1 ->  50  Spin S                 1.00
```

```
                State Dipole           15.2113
                Oscillator Strength     0.0000
                State Dipole Components   -9.6315   11.7717    0.2130
                Transition Dipole Components -0.0000  0.0000  -0.0000

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    61            0.574034
                    53  -->    62           -0.354528

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    61            0.574034
                    53  -->    62           -0.354528


  50 (Transition) Excitation Energy         165.7 nm
                                          60361.8 1/cm

       1 ->  51  Spin S                 -0.00
                State Dipole           15.1931
                Oscillator Strength     0.0003
                State Dipole Components   -9.5438   11.8197    0.2032
                Transition Dipole Components -0.0183  0.0124  -0.0975

       Spin Up :  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    61           -0.572243
                    53  -->    62            0.358315

      Spin Down:  Occ. MO  -->  Unocc. MO  Coefficients
      ------------------------------------------------
                    53  -->    61            0.572243
                    53  -->    62           -0.358315


**********************************************************************
******

Energy=-18421.610035 Gradient=193.849191 Symmetry=C1

ENERGIES AND GRADIENT
Total Energy                       = -102375.0617709 (kcal/mol)
Total Energy                       =  -163.141788254 (a.u.)
Binding Energy                     =  -18421.6100351 (kcal/mol)
Isolated Atomic Energy             =  -83953.4517358 (kcal/mol)
Electronic Energy                  = -584860.5621504 (kcal/mol)
Core-Core Interaction              =  482485.5003795 (kcal/mol)
CI Energy                          =       0.0000000 (kcal/mol)
Number of Configurations Used      =              51
Heat of Formation                  =  -14053.8400351 (kcal/mol)
Gradient of Reference Configuration =     193.8491915 (kcal/mol/Ang)
```

MOLECULAR POINT GROUP
  C1

EIGENVALUES OF THE REFERENCE CONFIGURATION(eV)

Symmetry:	1 A	2 A	3 A	4 A	5 A
Eigenvalue:	-61.304966	-52.479424	-50.443546	-48.529068	-46.769363

Symmetry:	6 A	7 A	8 A	9 A	10 A
Eigenvalue:	-43.962723	-42.119900	-41.014301	-39.410393	-36.170040

Symmetry:	11 A	12 A	13 A	14 A	15 A
Eigenvalue:	-35.181664	-34.990940	-34.122169	33.514561	-32.144753

Symmetry:	16 A	17 A	18 A	19 A	20 A
Eigenvalue:	-31.727345	-28.930212	-28.754284	-27.197771	-26.182789

Symmetry:	21 A	22 A	23 A	24 A	25 A
Eigenvalue:	-25.654743	-25.010000	-24.148544	-23.823021	-23.179008

Symmetry:	26 A	27 A	28 A	29 A	30 A
Eigenvalue:	-22.414204	-21.763659	-21.558001	-21.547756	-21.000868

Symmetry:	31 A	32 A	33 A	34 A	35 A
Eigenvalue:	-20.089334	-19.912460	-19.750763	-19.550419	-19.036018

Symmetry:	36 A	37 A	38 A	39 A	40 A
Eigenvalue:	-18.887974	-18.579193	-18.282911	-18.049023	-17.722288

Symmetry:	41 A	42 A	43 A	44 A	45 A
Eigenvalue:	-17.287371	-17.138041	-16.799072	-16.490490	-16.176798

Symmetry:	46 A	47 A	48 A	49 A	50 A
Eigenvalue:	-16.038591	-15.517140	-15.280633	-14.754498	-14.682483

Symmetry:	51 A	52 A	53 A	54 A	55 A
Eigenvalue:	-14.012991	-13.352755	-12.686942	-12.527109	-12.019317

Symmetry:	56 A	57 A	58 A	59 A	60 A
Eigenvalue:	-11.868505	-10.278853	-4.492475	-3.780133	-3.269904

Symmetry:	61 A	62 A	63 A	64 A	65 A
Eigenvalue:	-2.585544	-2.333029	-1.800455	-0.929585	-0.861136

Symmetry:	66 A	67 A	68 A	69 A	70 A
Eigenvalue:	-0.818001	-0.617029	-0.336741	-0.185313	-0.134149

Symmetry:	71 A	72 A	73 A	74 A	75 A
Eigenvalue:	0.123394	0.361328	0.492120	0.779032	0.911911

Symmetry:	76 A	77 A	78 A	79 A	80 A
Eigenvalue:	0.960716	1.217651	1.732971	1.911359	2.218365

```
Symmetry:     81 A         82 A         83 A         84 A         85 A
Eigenvalue:   2.520534    2.725586    2.900362    3.258740     3.402930

Symmetry:     86 A         87 A         88 A         89 A         90 A
Eigenvalue:   3.424565    3.520794    3.631697    3.783182     4.317157

Symmetry:     91 A         92 A         93 A         94 A         95 A
Eigenvalue:   4.870071   4.940001    5.313831     5.576722     6.262230

Symmetry:     96 A         97 A         98 A         99 A        100 A
Eigenvalue:   6.502975    7.078451    7.735449    7.908369     8.120407

Symmetry:    101 A        102 A        103 A        104 A        105 A
Eigenvalue:   8.380394    8.749744    9.291595    9.571287     9.823945

Symmetry:    106 A        107 A        108 A
Eigenvalue:   10.694991    11.597894    12.382694
```

```
ATOMIC ORBITAL ELECTRON POPULATIONS
AO:   1   S  C     1  Px  C     1  Py  C     1  Pz  C     2   S  C
          1.087656     0.965193     0.993249     0.944930     1.086817

AO:   2  Px  C     2  Py  C     2  Pz  C     3   S  C     3  Px  C
          1.002989     0.951856     0.955206     1.089776     0.979939

AO:   3  Py  C     3  Pz  C     4   S  C     4  Px  C     4  Py  C
          0.947242     1.025247     1.089561     0.953653     0.986437

AO:   4  Pz  C     5   S  C     5  Px  C     5  Py  C     5  Pz  C
          0.983483     1.041129     0.970483     0.970604     0.995842

AO:   6   S  C     6  Px  C     6  Py  C     6  Pz  C     7   S  C
          1.001910     0.895936     0.974286     1.056477     1.097265

AO:   7  Px  C     7  Py  C     7  Pz  C     8   S  C     8  Px  C
         0.987476     0.958340     0.933955     1.018870     0.961122

AO:   8  Py  C     8  Pz  C     9   S  C     9  Px  C     9  Py  C
          0.964486     1.022468     1.000525     0.975255     0.894684

AO:   9  Pz  C    10   S  N    10  Px  N    10  Py  N    10  Pz  N
          1.088962     1.136932     1.140653     1.204970     1.452780

AO:   1   S  C    11  Px  C    11  Py  C    11  Pz  C    12   S  C
          0.938071     0.855342     0.865895     1.072333     1.006718

AO:   12  Px  C    12  Py  C    12  Pz  C    13   S  N    13  Px  N
          0.964790     0.917233     1.037779     1.129607     1.157358

AO:   13  Py  N    13  Pz  N    14   S  C    14  Px  C    14  Py  C
```

```
          1.197652     1.439499     1.051801     0.879992     0.979822

AO:   14  Pz  C    15   S  C    15  Px  C    15  Py  C    15  Pz  C
          1.032817     1.088056     0.951668     0.992558     0.962023

AO:   16   S  C    16  Px  C    16  Py  C    16  Pz  C    17   S  C
          1.089164     0.989430     0.966958     0.933747     1.084412

AO:   17  Px  C    17  Py  C    17  Pz  C    18   S  N    18  Px  N
          0.986630     0.942439     0.994670     1.201977     1.843275

AO:   18  Py  N    18  Pz  N    19   S  C    19  Px  C    19  Py  C
          1.186456     1.097931     1.051513     0.995897     0.971977

AO:   19  Pz  C    20   S  C    20  Px  C    20  Py  C    20  Pz  C
          0.899618     1.051176     0.989065     0.985906     0.893726

AO:   30   S  C    30  Px  C    30  Py  C    30  Pz  C    31   S  O
          1.006426     0.864561     0.930418     0.712003     1.692722

AO:   31  Px  O    31  Py  O    31  Pz  O    33   S  O    33  Px  O
          1.229178     1.503841     1.884758     1.809625     1.957204

AO:   33  Py  O    33  Pz  O    24   S  H    25   S  H    26   S  H
          1.292002     1.488986     0.951177     0.968058     0.959111

AO:   27   S  H    28   S  H    29   S  H    21   S  H    22   S  H
          0.955726     0.950784     0.932402     0.946136     0.946005

AO:   32   S  H    23   S  H    34   S  H    35   S  H    36   S  H
          0.751575     0.946194     0.979248     0.967174     0.981319

AO:   37   S  H    38   S  H    39   S  H
          0.966679     0.982458     0.978607
```

NET CHARGES AND COORDINATES

Atom	Z	Charge	Coordinates(Angstrom)			Mass
			x	y	z	
1	6	0.008971	-0.28319	0.19238	0.38131	12.01100
2	6	0.003132	0.90546	-0.52107	0.31875	12.01100
3	6	-0.042204	2.10560	0.17493	0.19087	12.01100
4	6	-0.013133	-0.23172	1.58206	0.31306	12.01100
5	6	0.021942	0.96971	2.29716	0.18373	12.01100
6	6	0.071391	2.19327	1.57667	0.12118	12.01100
7	6	0.022964	2.26143	4.30176	-0.02417	12.01100
8	6	0.033054	1.00738	3.70463	0.11197	12.01100
9	6	0.040574	3.35802	3.45212	-0.06948	12.01100
10	7	0.064665	3.30535	2.18957	0.00241	14.00700
11	6	0.268358	4.51695	1.80238	-0.07110	12.01100

12	6	0.073480	4.68785	3.83609	-0.20042	12.01100
13	7	0.075883	5.33944	2.75786	-0.19329	14.00700
14	6	0.055569	6.60030	2.75400	-0.29637	12.01100
15	6	0.005695	7.34125	3.93420	-0.42431	12.01100
16	6	0.020701	6.65285	5.14944	-0.43645	12.01100
17	6	-0.008150	5.26229	5.10117	-0.31867	12.01100
18	7	-0.329639	4.95853	0.52275	-0.02124	14.00700
19	6	0.080996	5.14621	-0.18436	-1.28484	12.01100
20	6	0.080128	5.36307	-0.00549	1.27896	12.01100
30	6	0.486591	-0.07047	4.53970	0.16051	12.01100
31	8	-0.310499	-1.34167	4.16131	0.28748	15.99900
33	8	-0.547817	0.08117	5.73871	0.08950	15.99900
24	1	0.048823	7.14732	1.79655	-0.28679	1.00800
25	1	0.031942	2.97923	-0.48397	0.14590	1.00800
26	1	0.040889	0.89267	-1.62370	0.36696	1.00800
27	1	0.044274	-1.24683	-0.33624	0.47980	1.00800
28	1	0.049216	-1.21544	2.06247	0.36609	1.00800
29	1	0.067598	2.41183	5.39142	-0.09358	1.00800
21	1	0.053864	4.66146	6.02533	-0.32149	1.00800
22	1	0.053995	7.18644	6.10972	-0.53521	1.00800
32	1	0.248425	-1.87268	4.97563	0.29385	1.00800
23	1	0.053806	8.44040	3.90790	-0.51340	1.00800
34	1	0.020752	6.10517	0.12465	-1.75852	1.00800
35	1	0.032826	5.17587	-1.28462	-1.11800	1.00800
36	1	0.018681	4.31198	0.03213	-1.99089	1.00800
37	1	0.033321	5.70673	-1.06024	1.18794	1.00800
38	1	0.017542	6.20144	0.59357	1.70138	1.00800
39	1	0.021393	4.51163	0.02323	1.99626	1.00800

Dipole (Debyes)	x	y	z	Total
Point-Chg.	6.955	-3.043	-0.436	7.604
sp Hybrid	0.308	0.092	-0.033	0.323
pd Hybrid	0.000	0.000	0.000	0.000
Sum	7.263	-2.951	-0.469	7.854

HyperChem log stop -- Mon Jul 01 20:44:27 2002.